A NEW TOXICANT OCCURRING NATURALLY IN CERTAIN SAMPLES OF PLANT FOODSTUFFS [1]

XI. THE EFFECT OF FEEDING TOXIC AND CONTROL FOODSTUFFS ALTERNATELY

KURT W. FRANKE

Department of Experiment Station Chemistry, South Dakota State College, Brookings

FIVE FIGURES

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In a previous publication the author (Franke, '35) reported the effect of feeding rats diets containing toxic foodstuffs in varying amounts and for different time periods. In the following paper is reported the effect of feeding alternately diets containing toxic and control corn to simulate the consumption of vegetation by grazing animals.

EXPERIMENTAL

The rats used in this experiment were of Wistar Institute origin and had been weaned at 21 days of age and maintained for 1 week on a stock diet. Litter mates were then divided into four groups of five each. The rats were weighed every 5 days, and the food intake was weighed daily. Each rat was kept in an individual cage (Burr and Burr, '29).

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The composition of the diets was as follows:

	Per cent
Ground whole corn	70.0
Casein	11.0
Sucrose	15.0
Lard	2.0
Calcium carbonate	1.4
Sodium chloride	0.6
Total	100.0

NOTE: One diet contained toxic corn laboratory no. 388, and the other contained control corn.

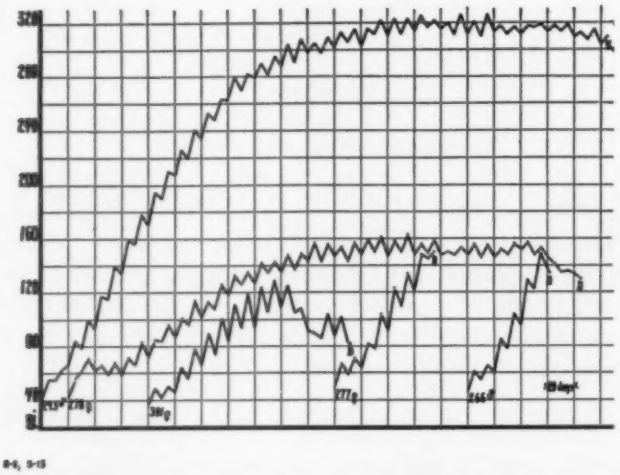

Fig. 1 Growth curves of group A, fed alternately for 5-day periods, starting with diet containing control corn, then diet with toxic corn.

Four groups of rats were fed as follows:

Group A	Fed alternating 5-day periods	Control and toxic diets
Group B	Fed alternating 5-day periods	Toxic and control diets
Group C	Fed alternating 10-day periods	Toxic and control diets
Group D	Fed alternating 15-day periods	Toxic and control diets

The growth curves for group A are shown in figure 1. The rhythmic gain and loss of weight correlate with the food intake. The first death in this group occurred on the sixty-

second day of experimentation (rat no. 266 M) while the last rat (no. 293 M) was killed on the four hundred and twenty-fifth day.

In group B only one death had occurred on the three hundred and second day (no. 302 F). The others were killed on the four hundred and twenty-fifth day. The same rhythmic response in growth (fig. 2) and food consumption took place.

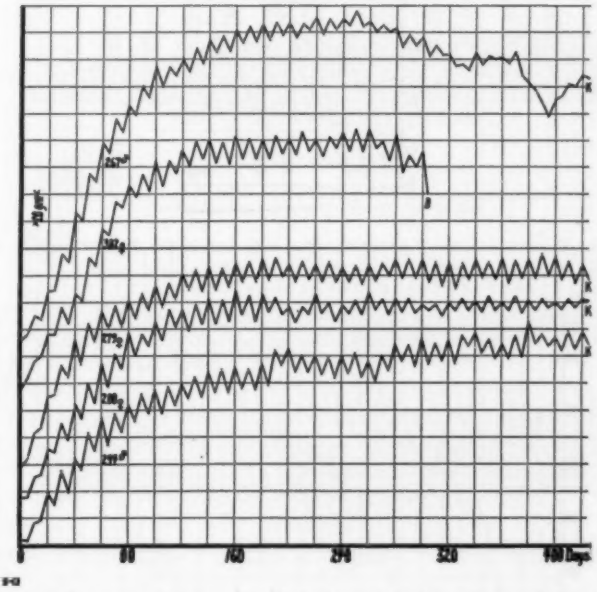

Fig. 2 Growth curves of group B. Fed alternately for 5-day periods, starting with diet containing toxic corn, then diet with control corn. Starting weights of the rats were: 294 M, 44 gm.; 280 F, 45 gm.; 279 F, 49 gm.; 302 F, 36 gm.; and 267 M, 42 gm.

Since these rats were all litter mates, it is difficult to explain why only one rat should have died in group B while four died in group A. The growth of the rats in group C (10-day alternation) is shown in figure 3. Only one rat lived to the one hundred and forty-fourth day. This group showed the same rhythmic response in growth and food consumption as was observed in the other groups.

The shortest span of life was in the 15-day alternating group (group D) as shown by rat 282 F (32 days), while one survivor was killed on the four hundred and twenty-fifth day.

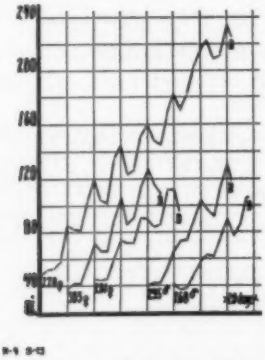

Fig. 3 Growth curves of group C. Fed alternately for 10-day periods, starting with diet containing affected corn, then diet with control corn.

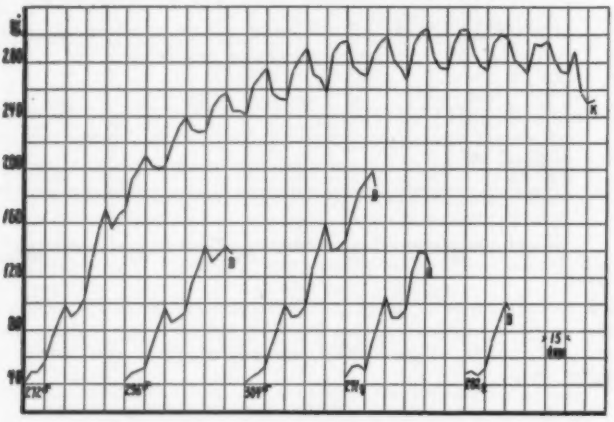

Fig. 4 Growth curves of group D. Fed alternately for 15-day periods, starting with diet containing affected corn, then diet with control corn.

A very rhythmic food consumption for this rat is shown in figure 4. The next longest survivor lived only to the ninety-seventh day.

The external appearances and gross pathological conditions varied from practically normal to the severe conditions previously described (Franke, '34).

The food consumption for the males is shown in figure 5. The food consumption of the females varied in exactly the same manner, but averaged slightly lower.

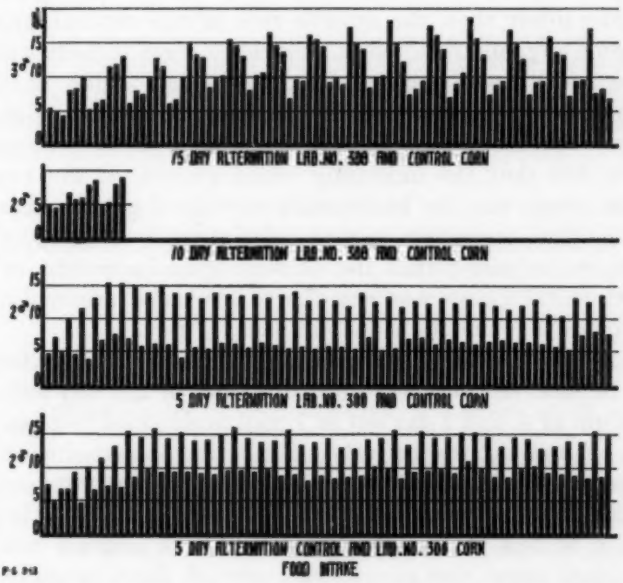
15 DAY ALTERNATION LAB.NO. 300 AND CONTROL CORN
10 DAY ALTERNATION LAB.NO. 300 AND CONTROL CORN
5 DAY ALTERNATION LAB.NO. 300 AND CONTROL CORN
5 DAY ALTERNATION CONTROL AND LAB.NO. 300 CORN
FOOD INTAKE

Fig. 5 Food intake of males in groups A, B, C and D, respectively.

DISCUSSION

In this series of alternating diets a rhythmic gain and loss of weight took place with a net gain on every change to control diet. This increased the body weight to a final weight approaching that normally obtained in controls on this diet. It is well to remember that an animal grazing might be in a position to alternate good with affected foods in shorter periods of time than those given in this series.

The only other reports in which similar rhythmic gain and loss of growth are shown are those in which forced periodic starvation occurred. Kopec and Latyszewski ('31 a, '31 b, '32 a, '32 b, '32 c) found that on an inadequate diet such as wheat, the control mice soon ceased to grow. The growth of the experimental group fed every other day was even lower. The growth rate of the experimental group on the days when fed was lower than the growth rate of the control rats on the same day, and resulted in a mean decrease in body weight. These results were obtained in two series. Both of these groups resumed growth when placed on an adequate diet, but the experimental groups in both series made the better growth. Kopec felt that the unusually rapid growth of the experimental group was due to a certain storage of growth capacity during the preceding period of intermittent starvation. Autopsies revealed that the unusual gain in weight of the experimental group was not due to abnormal accumulations of food or excrements in the alimentary tract.

In another series, during the first period of experimentation, he starved groups of mice 1 day out of 2, 1 day out of 3, 1 day out of 5, and 1 day out of 7, and designated them as '½,' '⅓,' '⅕' and ⅐,' respectively. By basing his conclusions on the growth during the first period he found that the negative changes in body weight on starvation days diminished in proportion to less intense starvation. Where animals were in individual cages the growth rate on fed days exceeded the control rate except in the case of the '½' group. This was not the case when four animals were in a cage, probably explained in that the greater activity of the animals overbalanced such a factor as increased warmth.

Robertson, Marston and Walters ('34) reported that intermittent starvation 2 days in every 7 resulted in an immediate 12 per cent body weight loss, which was regained on access to food. The mean body weights of the starved animals became greater than those of the controls after both had been fed freely.

It appears, on comparing the growth of the surviving rats in the alternately fed groups with the controls of other series, as if a similar higher mean weight was obtained by alternate feeding. This is of such interest that further studies should be carried out, and if possible the factor which is definitely involved should be determined.

CONCLUSION

The alternate feeding of toxic diets and control diets gave growth and food consumption curves of rhythmic decreases and increases in all groups when the diets were changed. The intervals were of 5-, 10- and 15-day alternations.

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THE IRON REQUIREMENT OF THE NORMAL HUMAN ADULT

GEORGE E. FARRAR, JR., AND S. MILTON GOLDHAMER
Thomas Henry Simpson Memorial Institute for Medical Research, University of Michigan, Ann Arbor

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Measurement of either the minimal or the optimal amount of iron necessary in the daily diet of the normal adult has seldom been attempted. Information concerning the normal daily iron requirement is urgently needed in the solution of problems arising in the recent widespread study of the anemias. The lack of extensive information concerning the quantitative metabolism of iron may well be explained by the difficulties encountered in the analysis of iron in biological materials, the small amount of iron metabolized by the human organism and the impossibility of obtaining a palatable iron-free or even a very low iron containing diet which is otherwise adequate for prolonged human consumption. At the University of Michigan Hospital normal young adults were eating accurately weighed diets during the course of insensible loss of weight experiments. These subjects have afforded a rare opportunity to observe the iron exchange in the human receiving a diet of low iron content.

PREVIOUS OBSERVATIONS

An estimate of the daily iron requirement was first sought in the determination of the amount of iron excreted by the fasting human. In the classical study by Lehman and his associates (1893), the iron excreted in the stool of Cetti during a 10-day fast was 7.3 mg. per day, and of Breithaupt during a 6-day fast 8.0 mg. per day. Urinary iron was not

considered in this study and the nature of the analytical method employed for the iron determinations was not described. Since the period of fasting was very short, the iron of the food present in the gastro-intestinal tract at the beginning of the fast must account for an appreciable amount of the stool iron observed. Furthermore, the loss of body tissue during fasting could account for some excretory iron which would not be excreted under normal dietary conditions. Benedict's ('15) study of a man fasting for a month unfortunately did not include measurement of the iron excreted.

Stockman (1895) analyzed the diets served in two Edinburgh hospitals and nurses' homes, as well as duplicate diets to those ordinarily eaten by himself and his friends. The iron content of these diets varied between 6 and 11 mg. per day. The daily food intake of two chlorotic girls was analyzed on 2 successive days. These extremely limited and inadequate diets varied between 1.3 and 3.2 mg. of iron per day. Stockman's analytical method involved ashing the entire diet and titrating the reduced solution of the ash with potassium permanganate. His analyses of the iron content of some common foods are similar to those obtained by Peterson and Elvehjem ('28). Stockman and Greig (1897) reported iron balance studies for the last 3 days of 8-day diet periods, using normal adult subjects. One person receiving 6.2 mg. of iron per day excreted 6.3 mg. daily. A young woman ingesting 3.5 mg. per day excreted 3.7 mg. In two other experiments the excretion greatly exceeded the intake. Stockman's values for urinary iron are likely much too high.

Von Wendt ('05) made an extensive study of the protein and salt requirement of a normal adult which is of little significance for iron metabolism because of the almost daily changes made in the diet which do not allow time for the gastro-intestinal tract to empty itself of the previous days' higher or lower iron containing diet, and because of the use of Neumann's wet ash iodometric analytical method whose gross inaccuracy was demonstrated by Lintzel ('28) in a critical study. Von Wendt's data show an increase in stool iron for several days following a day with a high iron intake.

A more satisfactory study was carried out by Sherman ('07). During 3 days, an adult male ate a diet of crackers and milk containing 1690 calories, 63 gm. of protein and 5.7 mg. of iron daily. The average stool iron during these 3 days was 5.3 mg. per day plus an average urine iron of 0.2 mg., a total of 5.5 mg. excreted per day, a positive balance of 0.2 mg. per day. In the other two experiments the subjects developed diarrhea on the third day and the excretion of iron as might be expected exceeded the intake. Considering the absence of an adjustment period of at least 3 days on this cracker and milk diet before the iron excretion was measured and the difficulties inherent in the volumetric permanganate method of iron analysis, this subject was roughly in iron balance with a daily intake of 5.7 mg. of iron.

This problem was reinvestigated by Lintzel ('29). He first ('28) studied various analytical methods for iron determination and was concerned particularly with the iron content of normal urine. He developed an ingenious but laborious method for determining small amounts of iron and reported that normal adult male urine contained less than 0.01 mg. of iron daily. Lanyar, Lieb and Verdino ('33) confirmed this absence of iron in normal urine with Lintzel's method. Lintzel ('29) obtained similar negative results in urine with the volumetric titanium method and later ('33) with the bipyridyl method. The accuracy of Lintzel's first method has been confirmed by Farrar in this laboratory, but the urine of five normal subjects has varied between 0.02 and 0.03 mg. of iron per 100 cc. which is about twenty times the amount found by Lintzel, and corresponds to the values reported by Sherman ('07), by Rose and her associates ('30) and by Hanzal and Bing ('34). Lintzel fed a normal young male subject a constant diet containing 13.8 mg. per day for 4 days. During these 4 days the stool iron averaged 14 mg. daily. During the next 6 days the subject received a constant diet consisting of boiled rice, butter (especially prepared), egg white, tea and sugar in amounts giving a total daily iron intake of 0.9 mg. During the last 3 days of this diet period

the stool iron averaged 0.87 mg. per day. During the following 6 days the subject ate the first diet again, containing 13.8 mg. of iron a day. During the last 3 days of this period the stool iron averaged 13.8 mg. per day. The total iron intake of the 16-day period was 143.4 mg.; the total stool iron was 143.7 mg. Similar experiments were carried out with diets containing 1.6, 2.1 and 3.6 mg. of iron per day. The stool iron always corresponded closely with the food iron, requiring 2 to 3 days after a change in intake to reach equilibrium. Lintzel further found in these normal subjects that large doses of inorganic iron led to retention only on the first day of their administration. The problem of absorption and reexcretion of iron by the gastro-intestinal tract is not within the realm of a balance experiment.

Davidson and his associates ('33) studied the iron intake of the Aberdeen poor. Although some of the women and children were anemic, there was no correlation between the incidence of anemia and the iron content of the diet including diets affording only 6 mg. of iron daily over long periods of time. Sherman's ('07) dietary survey of various sections of the United States revealed a variation of daily iron intake from 7 mg. for the Alabama negro to 31 mg. in the Northern lumber camps.

The recent studies of Elvehjem, Hart and Sherman ('33) and of Sherman, Elvehjem and Hart ('34), concerning the availability of iron in many foods (the proportion of the total iron content of a food which the gastro-intestinal tract and the organism is able to utilize) has added a most important point of view in the study of iron metabolism. The total iron content of the diet is of less importance than the amount of iron actually available for the use of the body in the foods employed.[1] On this basis subject 'W' (described below) was

[1] The availability of the total iron content of some foods studied by simultaneous chemical (bipyridyl) and biological (milk anemia in young rats) methods in Elvehjem's laboratory is as follows: beef liver 70 per cent; beef heart 70 per cent; beef steak 50 per cent; wheat 50 per cent; oats 50 per cent; oysters 25 per cent; spinach 20 per cent. Hematin iron is not used by the gastro-intestinal tract.

receiving about 3 mg. of available iron a day when the total iron of the diet was 5 mg.

Several studies of the iron requirement of the infant and growing child have appeared, but this paper is not concerned with growth or abnormal aspects of iron metabolism.

EXPERIMENTAL

Except for short periods no satisfactory measurements of the iron requirement of the normal adult are available. At the University Hospital, Drs. L. H. Newburgh and F. H. Lashmet have been carrying out insensible loss of weight measurements on young adults under very carefully controlled conditions over periods of several months' duration. Because of their accurately measured, constant, simple diets, such subjects are ideal for a study of iron exchange. Precautions against iron contamination were instituted in the regime of four of these subjects. The details of this procedure which measures the total caloric exchange in such normal subjects living their usual active lives were reported by Newburgh, Wiley and Lashmet ('31). The iron content of the foods in use was determined. Toward the end of the period of observation the iron excreted in the urine and stool was measured and the usual routine blood examinations were performed, including blood iron determinations.

Incident to these observations an extensive study of the accuracy of analytical methods for iron determination in biological materials has been carried out by Farrar ('35). The thiocyanate analytical method described by Stugart ('31) as modified by Elvehjem ('34) was adopted in this laboratory. Analyses were performed in triplicate; iron was added to one of the three samples to test its recovery.[2]

A restricted number of simple foods were used in varying amounts. The constituents of the diet for each day were accurately weighed on a beam balance (sensitivity 0.1 gm.)

[2] Since the accuracy of the analytical method is ±0.1 mg., the differences observed between the amounts of iron ingested and excreted are within the limits of error of the method.

individually, and then collectively as a check, by Doctor Newburgh or Doctor Lashmet, each morning. This ration was placed in a refrigerator and consumed by the subject in two or three portions. The milk and cream were obtained daily from the local dairy. Repeated analyses showed a surprising constancy of the iron content. The other foods were obtained in large lots. One subject (W) drank distilled water, the other three tap water, from individual thermos jugs which were weighed daily. This tap water contains an appreciable amount of iron which is included in the calculated iron intake. All of these subjects received a maintenance number of calories and were in nitrogen balance. Contamination of the excreta with iron was avoided by the use of new unchipped enamel ware.

Blood iron was determined by Hanzal's ('33) method. Hemoglobin was measured with the Sahli method using instruments standardized by the oxygen capacity method. Bureau of Standards instruments were used for the red and white blood cell counts. The menstrual hemoglobin loss was ascertained by the following rough method: Extract the napkins repeatedly with cold water in the ice box until the extractions are colorless, filter, treat an aliquot portion with dilute hydrochloric acid, compare in a colorimeter with an acid hematin solution of known concentration. Obviously such a method produces only a low approximation of the blood discharged.

Table 1 shows the iron content of the foods employed.

Tables 2, 3, 4 and 5 show for each subject the daily diet and the daily iron intake by periods as well as the stool, urine and blood iron where these were measured.

Table 6 summarizes the findings on all four cases.

Subject W, a male graduate student 26 years of age, lived on this regime for 316 days. For this entire period the average daily iron intake was 4.9 mg. During the last 31 days of this period the iron excretion was measured. The daily intake during this month averaged 5.2 mg., the stool iron averaged 5.2 mg. per day. In our experience normal male urine

has contained about 0.02 mg. of iron per 100 cc. using several analytical methods. At the end of 10 months on a diet containing a daily average of 4.9 mg. of iron this subject remained in iron balance. Furthermore, the blood iron, hemoglobin and red blood cell levels were within the normal range at the end of this period of low iron intake.

Subject V, a male graduate student 23 years of age, lived on this regime for 160 days. The average iron intake was 7.8 mg. per day. The urine and stool excreted for 5 days during the last month of this 5-month period were analyzed.

TABLE 1

Iron content of the food samples used in these diets

FOOD	IRON
	mg. per 100 gm. as fed
Milk, whole, pasteurized	0.05
Cream, 40%	0.10
Cheese, Cheddar	0.80
Bread, white	0.22
Bread, whole wheat	1.75
Shredded wheat	4.00
Puffed wheat	4.10
Bran, washed	8.50
Butter	0.19
Jelly, grape	1.00
Apple sauce, canned	0.29
Grape fruit, canned	0.70
Tomato juice, canned	1.30
Ann Arbor tap water	0.08

The subject was in iron balance on an iron intake of 7.7 mg. per day. Like the first subject, the blood findings were normal.

Subject J was a patient 18 years of age with an arrested minimal pulmonary tuberculosis, who was continued on bed rest during this study. His diet averaged 7.1 mg. of iron per day for 4 months. The intake and excretion of iron were equal during 5 days of the last month of this study and there was no anemia at the beginning or the end of this 4-month period.

TABLE 2

Subject W daily diet

DATES		2–29 to 3–24	3–25 to 5–4	5–5 to 6–29	6–30 to 7–14	7–15 to 8–31	9–1 to 9–11	9–12 to 10–13	10–14 to 10–27	10–28 to 12–3	12–4 to 12–12	12–13 to 12–29	12–30 to 1–12
Diet		2	3	4	5	6	7	8	9	10	11	12	13
Milk, whole	(gm.)	1900	1900	1900	1900	3032	2965	1500	1530	1500	1473	1610	1643
Cream, 40%	(gm.)	50	40	140	140	70	170	300	250	300	340	280	230
Bread, white	(gm.)	200	190	190	190	190	190	0	0	0	0	0	0
Bread, whole wheat	(gm.)	0	0	0	0	0	0	150	150	150	150	60	60
Butter	(gm.)	40	30	50	50	35	35	0	0	0	0	10	10
Jelly	(gm.)	0	0	0	0	0	0	96	100	96	80	0	0
Sugar	(gm.)	0	0	25	25	25	25	0	15	0	10	15	15
Shredded wheat	(gm.)	24	24	24	24	24	24	0	0	0	0	48	48
Grape fruit, canned	(gm.)	200	200	200	200	200	200	200	200	163	163	163	163
Bran, washed	(gm.)	0	5	5	5	5	5	0	0	0	0	0	0
Total iron intake per day	(mg.)	3.9	4.2	4.4	4.4	5.5	4.9	6.0	6.0	5.8	5.6	5.2	5.2
Fecal iron per day	(mg.)											5.4	5.0
Urine iron per day	(mg.)											0.2	0.2
Blood iron	(mg. %)											56.3	

TABLE 3

Subject J daily diet

DATES		10–11 to 11–24	11–25 to 12–4	12–5 to 12–14	12–15 to 12–21	12–22 to 12–23	12–24 to 1–8	1–9 to 1–18	1–19 to 1–28
Diet		A	B	C	D	E	F	G	H
Milk, whole	(gm.)	1000	1021	1053	1021	934	1000	1700	1700
Cream, 40%	(gm.)	220	188	140	188	188	150	100	200
Bread, whole wheat	(gm.)	150	150	150	150	180	150	150	150
Butter	(gm.)	50	50	50	50	84	60	10	40
Shredded wheat	(gm.)	24	24	24	24	24	0	0	0
Grape fruit, canned	(gm.)	100	100	100	100	100	0	0	0
Jelly, grape	(gm.)	90	90	90	90	10	0	0	0
Sugar	(gm.)	25	14	13	14	14	10	10	10
Bran, washed	(gm.)	5	5	0	5	0	0	5	0
Puffed wheat	(gm.)	0	0	0	0	0	10	10	10
Tomato juice (canned)	(gm.)	0	0	0	0	0	100	100	100
Apple sauce, canned	(gm.)	0	0	0	0	0	150	100	100
Cheese, Cheddar	(gm.)	0	0	0	0	0	30	60	50
Tap water	(gm.)	1000	1000	1000	1000	1000	1000	1000	1000
Total iron intake per day	(mg.)	7.2	7.2	6.8	7.2	6.5	6.8	7.2	7.3
Stool iron per day	(mg.)								7.2
Urine iron per day	(mg.)								0.2
Blood iron	(mg. %)								54.0

TABLE 4
Subject V daily diet

DATES		9–22 to 9–30	10–1 to 10–18	10–19 to 10–28	10–29 to 12–7	12–8 to 12–27	12–28 to 1–21	1–22 to 1–31	2–1 to 2–10	2–11 to 3–2
Diet		A	B	C	D	E	F	G	H	I
Milk, whole	(gm.)	1000	1000	1099	1050	1030	1030	600	710	677
Cream, 40%	(gm.)	200	200	50	125	155	184	200	300	350
Bread, whole wheat	(gm.)	200	200	200	200	200	230	200	140	140
Butter	(gm.)	60	60	60	60	60	70	50	40	40
Shredded wheat	(gm.)	48	48	48	48	48	0	0	0	0
Grape fruit, canned	(gm.)	100	100	100	100	100	150	150	150	150
Jelly, grape	(gm.)	90	90	90	90	90	100	100	100	100
Sugar	(gm.)	10	20	20	20	8	10	0	0	0
Bran	(gm.)	0	0	5	5	5	0	0	0	0
Puffed wheat	(gm.)	0	0	0	0	0	10	10	10	10
Apple sauce, canned	(gm.)	0	0	0	0	0	0	0	0	150
Tap water	(gm.)	500	500	500	500	500	500	500	500	500
Total iron intake per day	(mg.)	8.2	8.2	7.6	8.6	8.6	7.7	7.0	6.1	6.5
Stool iron per day	(mg.)						7.6			
Urine iron per day	(mg.)						0.2			
Blood iron	(mg. %)							52.8		

Subject R was a dietitian 24 years of age who lived on this regime for 41 days with an average iron intake of 9.1 mg. per day. She likewise was in iron balance and showed normal blood iron and hemoglobin levels. Her blood was examined

TABLE 5
Subject R daily diet

DATES		1–8 to 1–24	1–25 to 2–17
Diet		A	B
Milk, whole	(gm.)	1200	1400
Bread, whole wheat	(gm.)	180	210
Shredded wheat	(gm.)	48	48
Jelly	(gm.)	65	85
Grape fruit, canned	(gm.)	200	300
Sugar	(gm.)	18	10
Tap water	(gm.)	825	825
Total iron intake per day	(mg.)	8.3	9.8
Stool iron per day	(mg.)	8.0	
Urine iron per day	(mg.)	0.2	
Blood iron	(mg. %)		47.8

TABLE 6
Summary of four subjects

SUBJECT	TOTAL NUMBER OF DAYS ON THE DIET	AVERAGE IRON INTAKE PER DAY	IRON BALANCE EXPERIMENT				BLOOD FINDINGS AT THE END OF THE WHOLE PERIOD	
					Output			
			Days	Intake of iron per day	Stool iron average per day	Urine iron average per day	Blood iron	Hemoglobin % (Sahli) 16.6 gm. = 100 %
		mg.		*mg.*	*mg.*	*mg.*	*mg.*	
W	316	4.9	31	5.2	5.2	0.2	56.3	98
V	160	7.8	5	7.7	7.6	0.2	52.8	94
J	110	7.1	5	7.3	7.2	0.2	54.0	97
R	41	9.1	17	8.3	8.0	0.2	47.8	80

tri-weekly throughout the 41 days. No significant changes occurred in the hemoglobin percentage and the red or white blood cell count. The amount of hemoglobin lost during a menstrual period which occurred during period 'B' of this

study was found to correspond to 33 cc. of the subject's blood or about 12.0 mg. of iron. A second dietitian, not included in this study, showed a similar hemoglobin loss. These figures correspond closely with those reported by Fowler and Barer ('35) for the amount of blood lost in a normal menstrual period. Because of the hemoglobin decomposition inherent in menstruation, as well as the decomposition occurring during the extraction process, and the other forms of iron present in the menstrual discharge (Ohlson and Daum, '35) our estimate of the amount of iron lost is certainly too low. Unfortunately the excreta during period 'B' were discarded before iron analyses were completed.

SUMMARY

1. A healthy male, 26 years of age, carried on his usual duties as a graduate student for 316 days on a diet whose average daily iron content was 4.9 mg. During the last 31 days of this period the subject was in iron balance when the diet contained 5.2 mg. of iron daily. The blood hemoglobin, blood iron and red blood cell levels were within normal limits.

2. Two other male subjects were likewise in iron balance after 4 and 5 months on diets containing 7.1 and 7.8 mg. of iron daily respectively, and their blood contained normal amounts of hemoglobin and red blood cells.

3. A young woman living for over a month on a diet containing 9.1 mg. of iron daily was in iron balance during the intermenstrual phase. The total menstrual blood loss represented 33 cc. of the subject's blood.

4. Normal urinary iron amounts to about 0.02 mg. per 100 cc. of urine.

5. These observations, together with those in the previous literature, indicate that the iron requirement of the normal adult male is not more than 5 mg. daily. The obvious importance of the availability of iron in the diet has only recently been demonstrated.

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METABOLISM IN THE RAT OF THE NATURALLY OCCURRING ARSENIC OF SHRIMP AS COMPARED WITH ARSENIC TRIOXIDE

E. J. COULSON
U. S. Bureau of Fisheries[1]

AND

ROE E. REMINGTON AND KENNETH M. LYNCH
Medical College of the State of South Carolina, Charleston

ONE FIGURE

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Beginning with the announcement of Couerbe (1834) to the French academy a little over 100 years ago that he had found arsenic in the putrid bodies of human beings, a controversy has existed as to whether or not arsenic is a normal constituent of the animal organism. With the development of more sensitive and more accurate methods for the detection and determination of arsenic, there is a growing body of evidence that minute traces of arsenic are of wide-spread occurrence in normal plant and animal tissues.

Billeter and Marfurt ('23) have examined the organs and tissues of seventeen cadavers, ranging from newborn to 70 years of age (without arsenical medication) and found arsenic always present, increasing in amount with age. Fordyce, Rosen and Meyers ('22) found arsenic to be present in normal urine in about 75 per cent of forty cases, and in normal blood in 50 per cent of 130 cases, while Guthman and Grass ('32) found arsenic always present in the venous blood of women, averaging 0.64 part per million. A very interesting feature,

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according to Guthman and Grass is that during menstruation the arsenic concentration of the blood rises by 50 per cent to an average of 0.93 part per million. During pregnancy, the increase is still greater, reaching its maximum of 2.20 parts per million between the fifth and sixth months. In the later months of pregnancy, there is a gradual fall, but even at full term the value is still above the inter-menstrual value. Guthman and Grass suggest that this increase in blood arsenic may be related to growth processes and cell proliferation. Godonnèche and Dastugue ('34) have also reported that the rate of growth and the date of metamorphosis of tadpoles are accelerated by solutions containing small amounts of arsenic.

The source of the arsenic in the urine, blood and organs of humans can readily be traced to the arsenic content of the dietary and water supply, particularly with the present day widespread use of arsenical sprays and of foods prepared by artificial processes. Nevertheless, Headden ('10) has reported the common occurrence of arsenic in virgin soils as well as in the plants growing thereon while the fact that sea foods contain arsenic is well known. It therefore seems evident that whether or not arsenic is of physiological importance to the body, it is always present.

Within the range of nutritive utilization, it has been assumed, without a great deal of experimental evidence, that mineral elements in the so-called 'natural' combination in which they occur in foods are better utilized than the inorganic salts. Evidence to the contrary, however, is accumulating. The iron of hematin, for example, is but poorly utilized (Elvehjem, '32) and that of meat and liver less readily than that of wheat (Rose, Vahlteich and MacLeod, '34), while for oysters, spinach, alfalfa and blood, only 25 per cent, or less, of the iron is available for hemoglobin building purposes (Sherman, Elvehjem and Hart, '34).

In the range of toxicity, arsenic trioxide, for example, is more toxic to higher animals than certain aromatic organic compounds of arsenic (the arsphenamines). It has also been shown that certain forms of copper as it occurs in foods and

the character of the diet which accompanies the feeding of inorganic copper salts may greatly influence the quantities of the metal which may be stored in the bodies of experimental animals (Coulson, Remington and Lynch, '34).

It is well known that practically all marine life is normally richer in arsenic than products from the land. Vertebrate sea fish from British and Swedish waters, according to Cox ('25), contain from 0.1 to 3.0 mg. arsenic (as As_2O_3) per kilogram, fresh basis. Chapman ('26) analyzed a large number of shellfish and crustaceans from the coastal waters of the British Isles. His values varied from a minimum of 3 mg. per kilogram in the case of an oyster sample to a maximum of 174 mg. per kilogram in one sample of prawns and he found the sea water, from which these fish were taken, to contain arsenic in concentrations ranging from 0.14 to 1.0 part per million.

The arsenic content of some American shellfish and crustaceans as reported by White ('33) is lower than those reported by Chapman, but the values for vertebrate fish are somewhat higher than those reported by Cox. The results of these investigators agree, however, in that crustaceans contain more arsenic than any other sea animals.

In the course of our analyses of various sea foods, it was noted that shrimp grown in certain localities may contain relatively large quantities of arsenic. These shrimp seem to grow and thrive in spite of an arsenic content much higher than that of most other marine organisms. Table 1 shows the arsenic content of samples of shrimp which have been collected from various localities during the course of about a year and a half. The concentration of arsenic varies in these shrimp from 1.27 mg. per kilogram to 41.60. This variation is probably due to the character and arsenic content of the vegetation on the feeding grounds in the localities in which these shrimp grow.

An interesting feature of the results shown in table 1 is the variation in arsenic content of shrimp collected from the same locality. Samples collected near the vicinity of the

mouth of the Stono River near Charleston, S. C., on October 25 and 28, 1933, contained, respectively, 152 and 171 mg. per kilogram of arsenic (dry basis); while samples collected during May and June of the following year contained only 21 and 51 mg. per kilogram. Again in October, 1934, the arsenic content of a sample of shrimp collected from the same locality was 137 mg. per kilogram. This variation in arsenic content

TABLE 1

Arsenic content of shrimp (edible portion) from various localities

DATE OF COLLECTION	SAMPLE NO.	FROM VICINITY OF	ARSENIC (As_2O_3) CONTENT	
			Fresh basis	Dry basis
			Milligrams per kilogram	
June 15, 1933	102[1]	Aransas Pass, Texas. Gulf shrimp	1.94	10.8
Sept. 25	116[1]	Aransas Pass, Texas. Bay shrimp	2.44	13.0
Aug. 15	107[2]	Savannah, Georgia	15.10	72.5
Aug. 15	108[2]	Savannah, Georgia	9.10	28.8
Sept. 9	113[2]	Mobile, Alabama	1.27	4.9
Oct. 13	117[2]	Lake Salvador near Bayou Barataria, Louisiana	18.80	98.4
Sept. 12	114[3]	Folly River near Charleston, S. C.	3.83	18.6
Sept. 26	115[3]	Off Folly Beach, S. C. Deep sea shrimp	17.30	82.0
Oct. 25	120[3]	Mouth of Stono River near Charleston, S. C.	36.60	152.0
Oct. 28	127[3]	Mouth of Stono River near Charleston, S. C.	41.60	171.0
May 7, 1934	150[3]	Mouth of Stono River near Charleston, S. C.	5.94	21.0
June 15	157[3]	Mouth of Stono River near Charleston, S. C.	15.40	50.6
Oct. 23	198[3]	Mouth of Stono River near Charleston, S. C.	30.70	137.0

[1] Canned shrimp.

[2] Peeled raw.

[3] Cooked and peeled.

of shrimp from the same locality suggests that there is some seasonal cause. Just what the significance of this may be or the source of the arsenic content of the shrimp from this particular locality is not known.

It is apparent that the availability of such a rich source of 'natural' or metabolized arsenic furnishes an excellent opportunity to study the relative metabolism, storage and toxicity of arsenic of these high arsenic containing foods as

against arsenic trioxide when fed to rats over a long period of time.

PLAN OF EXPERIMENT

Young rats of approximately 33 to 35 days of age were divided into five groups of sixteen animals each. The sexes were evenly divided in each group and were caged separately in large group cages with raised screen bottoms. Distilled water was given ad libitum.

The basic diet used was the Sherman diet 13 as modified by Russell, and has been described elsewhere (Levine, Remington and Culp, '31). In this investigation the usual proportions of the constituents were shifted slightly and had the following composition:

	Per cent
Ground whole wheat	59.3
Dried whole milk (Klim)	29.5
Meat and bone scraps	10.0
Sodium chloride	1.2
	100.0

In the shrimp diets the meat and bone scraps of the above described diet were replaced by dried shrimp. Two lots of dried shrimp were used; one lot (low arsenic shrimp) contained 10.4 mg. per kilogram of arsenic, and the other (high arsenic shrimp) contained 171 mg. per kilogram. The arsenic trioxide was added to the diets in the following manner: to 1 kg. of ground wheat in a large evaporating dish was added a solution consisting of 85 cc. of As_2O_3 in water (1 cc. = 2 mg. As_2O_3) to which was added 200 cc. of alcohol to facilitate mixing in the diet. This was mixed thoroughly and dried on a steam bath. After the addition of a sufficient amount of untreated ground wheat to bring the total weight up to the original 1 kg. (replacing the moisture which had been driven off), the wheat was reground and mixed thoroughly. The calculated amount of this arsenic wheat was then added to the basic diets, replacing an equivalent amount of untreated wheat.

The above described diets with their arsenic contents in milligrams per kilogram (As_2O_3), as determined by the method of the Association of Official Agricultural Chemists ('30), are shown in table 2.

The diets were fed for a period of 12 months. At intervals of 3 months four representative animals from each group were killed and histological examinations made on the liver,

TABLE 2

Arsenic content (As_2O_3) of arsenic fed rats

	WEEKS	STOCK DIET	LOW ARSENIC SHRIMP DIET	HIGH ARSENIC SHRIMP DIET	STOCK DIET AND As_2O_3	LOW ARSENIC SHRIMP DIET AND As_2O_3
		Milligrams per kilogram				
Arsenic content of diets		0.20	1.20	17.70	17.90	17.90
		Milligrams per rat				
Total arsenic ingested	13	0.24	1.29	19.23	19.78	19.60
	24	0.46	2.49	36.26	36.32	36.27
	39	0.72	3.86	56.95	57.53	58.22
	52	0.95	5.42	75.53	76.11	75.98
Total arsenic stored	13	....	0.11	0.13	3.73	3.57
	24	0.067	0.18	0.26	3.58	4.25
	39	0.088	0.15	0.30	4.09	4.59
	52	0.178	0.25	0.29	3.99	4.46
	54[1]	....	0.23	0.16	3.13	2.95
Percentage of intake stored by rats	13	....	8.5	0.7	18.9	18.2
	24	14.6	7.2	0.7	9.9	11.7
	39	12.2	3.9	0.5	7.1	7.9
	52	18.7	4.6	0.4	5.2	5.9

[1] Arsenic feeding was discontinued for 2 weeks.

spleen and kidney. The remaining portions of the livers of each group were then pooled and analyzed for arsenic. The female rats of each group after the removal of the alimentary canal were passed through a food chopper and also analyzed for arsenic.

Accurate records of food consumption were kept on all groups for the purpose of calculating the amount of arsenic consumed and to observe whether the incorporation of arsenic

in the diet affected appetite or utilization, such an effect having been claimed by Sollman ('21).

RESULTS

The food intake and rate of growth of the rats in the various groups were practically the same. The growth curves for

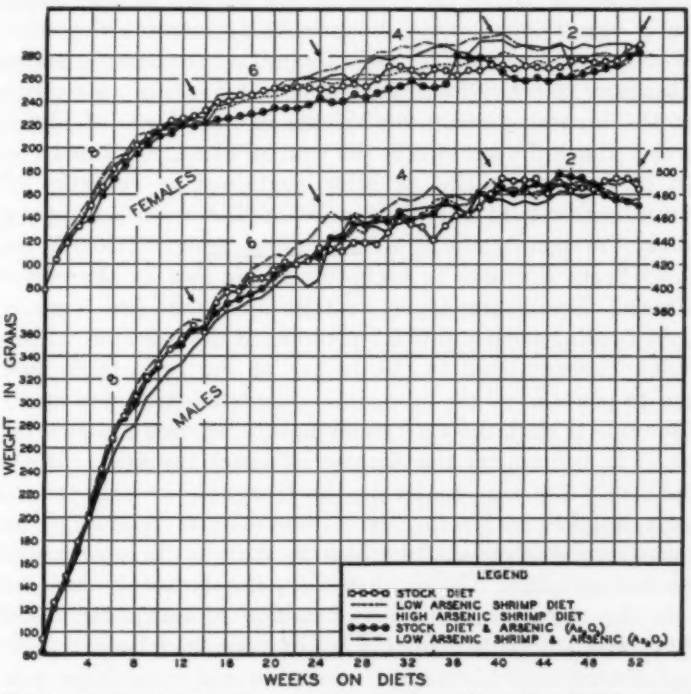

Fig. 1 Showing growth rates of rats on diets of varying arsenic content derived from shrimp and from added arsenic trioxide. Arrows indicate point at which rats were killed for analysis and figures between arrows represent number of animals in each group during that portion of the curve.

the rats on the above described diets are shown in figure 1. For the purpose of conserving space, the food intake data are not shown; however, if desired, they may be calculated from the data given in table 2.

Table 2 shows the total amount of arsenic which was ingested by the rats as well as the total amount and percentage of the arsenic from the various diets which were stored by the rats between the time intervals at which animals were sacrificed for analysis. It can be seen from this table that the arsenic content of rats on the stock diet slowly but continually increased during the experimental period. This observation is in agreement with the previously mentioned work of Billeter and Marfurt ('23), who found that the arsenic content of humans increased with age. The carcasses of rats receiving the largest amount of arsenic in the form of shrimp (ninety times the amount in the control diet), contained from two to four times the quantity contained in controls. In marked contrast to this, the rats which had received approximately the same quantity of arsenic as arsenic trioxide contained from fifty-five to sixty-five times that in the control animals. The results also show that during the first 3 months of the feeding period only 0.7 per cent of the ingested 'shrimp arsenic' accumulated in the bodies of the rats, while more than 18 per cent of the inorganic arsenic trioxide was stored. After this period, the rats receiving the 'shrimp arsenic' continued to accumulate the element at about the same rate but apparently the rats receiving the inorganic arsenic trioxide had, some time within the first 3 months of the feeding period, reached an equilibrium in which no more storage of arsenic was taking place. It is, therefore, impossible to calculate from the above results the percentage of inorganic arsenic which was stored before this equilibrium had been reached. Undoubtedly the percentage stored would have been much higher had the first feeding period been of shorter duration.

In table 3 is shown the concentration of the arsenic in the livers of the animals on the various diets. Here again it can be seen that the animals receiving inorganic arsenic were able to store it in concentrations of nearly 100 times the 'normal' amounts, while the 'shrimp arsenic' was stored only to the extent of about seven times the 'normal' concentration. These results also show how arsenic accumulates in the liver in

greater concentrations than in the rest of the body. Thus livers of rats receiving the low arsenic-shrimp diet plus As_2O_3 at the end of 13 weeks contained 57.7 mg. of arsenic per kilogram of fresh tissue, while the rest of the carcass contained only 14.9 mg. per kilogram. The liver, which consisted of only 5 per cent of the weight of the whole organism, contained 16 per cent of the arsenic.

After the rats had been on the arsenic diets for 1 year, arsenic feeding was discontinued for 2 weeks in order to obtain some idea of the rapidity with which metabolized arsenic is eliminated from the body. At the end of this time

TABLE 3

Arsenic content (As_2O_3) of livers of arsenic fed rats

WEEKS	STOCK DIET	LOW ARSENIC SHRIMP DIET	HIGH ARSENIC SHRIMP DIET	STOCK DIET AND As_2O_3	LOW ARSENIC SHRIMP DIET AND As_2O_3
	Milligrams per kilogram fresh tissue				
13	0.43	1.71	3.13	52.30	57.70
24	0.72	2.48	3.41	60.10	71.40
39	0.83	2.20	3.94	56.50	61.20
52	0.92	2.21	2.82	49.10	56.60
54[1]	....	2.10	2.47	38.30	41.80

[1] Arsenic feeding was discontinued for 2 weeks.

the remaining rats in each group were killed and analyzed for arsenic. The results shown in tables 2 and 3 indicate that about one-fourth to one-third of the arsenic is eliminated in this length of time.

There was no observable effect of the added arsenic on the physical vigor or appearance of any of the experimental animals. All of the rats appeared to be in healthy condition and, on autopsy, no gross abnormality of the viscera was observed. No histological evidence of injury to the spleen, liver or kidney could be detected when sections of these organs were compared with similar sections from the normal control animals in this series and in a previous large series of animals (Coulson, Remington and Lynch, '34).

ARSENIC EXCRETION EXPERIMENTS

Excretion experiments were conducted on rats and on two human subjects in order to determine whether or not the arsenic compound of shrimp is absorbed from the gastrointestinal tract and to obtain some information upon the relative rate of excretion of 'shrimp arsenic' as compared to the inorganic form.

Four adult rats were selected from our stock colony and divided into two pairs. The animals were placed in cages designed to separate the urinary and fecal material, the arrangement consisting of a round galvanized iron wire cage suspended within a large glass funnel below the stem of which was suspended a glass ball in such a manner that the urine flowed around the ball and dropped through a small funnel into an Erlenmeyer flask. The feces, upon striking the glass ball, were deflected into a large beaker provided for that purpose. The urine and feces were collected daily. The funnel was washed each day with hot distilled water and the washings, containing the small amount of scattered food and some urine which had dried upon it, were added to the urinary material.

The urinary and fecal arsenic was determined daily for 1 week in order to establish a value for the normal daily excretion of the rats on the stock diet. At the end of this time, the rats were given the high arsenic containing diet for 1 day and then the stock diet until the excretion decreased to the normal value.

The high arsenic-shrimp diet contained 13.7 mg. per kilogram of arsenic in the form of the natural compound of shrimp and the stock-diet-plus-arsenic-trioxide was made up to contain the same concentration.

Results of rat experiments

The results of the excretion experiments are shown in table 4.

The average daily arsenic excretion of the rats on the stock diet was 5.9 γ per day (γ ≏ 0.001 mg.) for group A and 6.5 γ

TABLE 4

Arsenic excretion in the rat

GROUP A							GROUP B					
Time in days	Diet	Arsenic ingested	Arsenic eliminated			Arsenic recovered	Diet	Arsenic ingested	Arsenic eliminated			Arsenic recovered
			Feces	Urine	Total				Feces	Urine	Total	
		γ	γ	γ	γ	Per cent		γ	γ	γ	γ	Per cent
	Stock		1.2	4.7	5.9		Stock		1.4	5.1	6.5	
1	Shrimp	480	14.1	350.0	364.1	74.6	As_2O_3	370	7.9	21.1	29.0	6.1
2	Stock		6.3	100.0	106.3	20.9	Stock		37.5	9.1	46.6	10.8
3	Stock		2.5	13.1	15.6	2.0	Stock		7.1	4.7	11.8	1.4
4	Stock		1.2	8.1	9.3	0.7	Stock		1.7	7.9	9.6	0.8
5	Stock		1.7	4.4	6.1		Stock		1.7	4.7	6.4	
6	Stock		1.9	4.2	6.1		Stock		1.8	5.1	6.9	
7	Stock		1.8	4.2	6.0		Stock		1.8	5.2	7.0	
					Total	98.2					Total	19.1
8	As_2O_3	425	18.1	37.0	55.1	11.6	Shrimp	425	3.9	324.0	327.9	75.6
9	Stock		26.2	6.3	32.5	6.3	Stock		3.9	85.0	88.9	19.4
10	Stock		5.3	12.5	17.8	2.8	Stock		1.4	12.7	14.1	1.8
11	Stock		1.6	5.5	7.1	0.3	Stock		1.4	8.1	9.5	0.7
12	Stock		1.5	4.7	6.2		Stock		1.5	5.1	6.6	
					Total	21.0					Total	97.5

for group B, about 80 per cent of the excreted arsenic being in the urine. The rats in group A were then given the high arsenic shrimp diet for 1 day and they consumed 35 gm. of the diet containing 480 γ of arsenic. It can be seen from the table that during the 24 hours that they were consuming the diet 74.6 per cent of the ingested arsenic appeared in the excreta and 98.2 per cent was recovered within 4 days, at the end of which time the arsenic excretion had fallen to the normal level. On the other hand, the rats in group B which had consumed 27 gm. of the diet containing 370 γ of inorganic arsenic had eliminated only 19.1 per cent of the ingested arsenic before the arsenic excretion had fallen to the normal level. In other words, 80 per cent of the ingested inorganic arsenic was retained in the bodies of the rats.

At the end of a week's time when it was certain that the arsenic excretion was proceeding at a normal rate, the experiment was repeated. This time group B received the shrimp diet and group A the inorganic arsenic. Again practically all of the ingested 'shrimp arsenic' was excreted in the first 4 days while 79 per cent of the inorganic arsenic was retained.

It is interesting to note that when the rats received arsenic in the form of shrimp about 96 per cent of the excreted arsenic appeared in the urine, but when inorganic arsenic was fed the excretion was about equally divided between the urine and feces. These results indicate that the arsenic compound of shrimp is very readily absorbed from the gastrointestinal tract but that it is rapidly and almost completely eliminated.

Excretion experiments on human beings

The results of rat experiments cannot always be applied to human beings, so in order to determine whether or not the excretion of arsenic is similar in these two subjects a similar experiment was conducted on two human subjects. No especial attempt was made to regulate the diet of the subjects during the course of the experiment with the exception that sea foods, which are known to contain arsenic, were avoided.

The result was that the normal arsenic excretion varied somewhat from day to day but it was felt that these variations would not be significant when compared to the relatively much larger amounts ingested and excreted when the shrimp sample was eaten.

TABLE 5

Arsenic excretion in human beings

Time in days	SUBJECT A					SUBJECT B				
	Arsenic ingested	Arsenic eliminated			Arsenic recovered	Arsenic ingested	Arsenic eliminated			Arsenic recovered
		Feces	Urine	Total			Feces	Urine	Total	
	γ	γ	γ	γ	Per cent	γ	γ	γ	γ	Per cent
	Normal	7	26	33		Normal	15	29	43	
1	1180	23	1014	1037	84.9	980	18	780	798	77.0
2		9	221	230	16.6		16	198	214	17.4
3	as	6	149	155	10.3	as	11	90	101	5.9
4	Shrimp	9	50	59	2.2	Shrimp	..	104	...	7.7
5							..	78	...	5.0
6							..	44	...	1.5
				Total	114.0				Total	114.5
13	1000	19	308	327	29.4	1000	31	340	371	32.8
14		..	200	....	17.4		..	258	...	22.9
15		..	108	....	8.2		..	140	...	11.1
16	as	..	102	....	7.6	as	..	150	...	12.1
17	As_2O_3	..	79	....	5.3	As_2O_3	..	122	...	9.3
18		..	69	....	4.3		..	98	...	6.9
19		..	41	....	1.5		..	108	...	7.9
				Total	73.7				Total	103.0

Results of excretion experiments on human beings

The results of the above described experiments are shown in table 5.

Subject A ate 200 gm. of boiled shrimp containing 1180 γ arsenic, while subject B ate 165 gm. containing 980 γ arsenic. From the results shown in table 5, it can be seen that the elimination of ingested 'shrimp arsenic' proceeded at about the same rate in the human subjects as in the rat. Inorganic arsenic, however, although excreted more slowly than 'shrimp

arsenic' is apparently eliminated more completely in human beings than in the rat.

In a somewhat similar experiment reported by Chapman ('26), a person ate 1 pound of lobster containing 33 mg. arsenic and he recovered 24.3 mg. or 74 per cent of the ingested arsenic in the urine within 48 hours at the end of which time the experiment was terminated. Fecal elimination was not determined.

DISCUSSION

It seems probable that the arsenic in the quantities in which it occurs in shrimp does not exist in a simple inorganic form but must be bound up into a more or less complex organic combination as the result of the metabolic processes of the organism. Whether this is an attempt on the part of the organism to diminish the toxicity of the arsenic or whether a certain proportion of the arsenic is actually found favorable to its development is, of course, impossible to say.

The results reported here, however, show that there is a difference in the metabolism of arsenic as it occurs in shrimp as compared with inorganic arsenic when fed to rats. That the arsenic from the shrimp is absorbed from the gastrointestinal tract is shown by our observation that after the ingestion of shrimp practically all of the ingested arsenic may be recovered in the urine within a relatively short time, indicating that following the normal process of digestion, the arsenic containing substance in shrimp yields a soluble and readily diffusible product which is not decomposed in the body and is rapidly eliminated by the kidneys.

Further evidence of the complexity of the arsenic in shrimp is seen in the difficulties encountered in its estimation by the usual sulfuric and nitric acid digestion and subsequent Gutzeit determination (Remington, Coulson and von Kolnitz, '34). It was shown that even after the destruction of practically all of the organic matter by treatment with sulfuric and nitric acids only negligible traces of the arsenic present could be determined in the Gutzeit flask, nor could the arsenic be pre-

cipitated out by magnesia mixture as described by Gross ('33) for the elimination of the interference due to pyridine. However, if the sulfuric acid digest of the shrimp were subjected to a higher temperature after the addition of $CuSO_4$ (as a catalyst), the complex arsenic compound was broken down and could be fully recovered in the Gutzeit determination.

The results of the experiments reported in this investigation are of interest not only for the light which they throw on the metabolism of arsenic but also as additional evidence that the manner in which inorganic elements are used in the body depends upon the source or form in which those elements are presented.

SUMMARY

1. Only a very small percentage of the arsenic from shrimp when fed to rats was stored in the animal organism, while inorganic arsenic when fed at the same level accumulated to the extent of fifty-five to sixty-five times the 'normal' concentration in the bodies of the animals and over 100 times the 'normal' concentration in the livers.

2. During the first 3 months of the feeding period 18 per cent of the ingested As_2O_3 was stored in the bodies of the rats (as against 0.7 per cent for 'shrimp arsenic') and the total quantity stored within the first 3 months was not significantly increased by feeding the element for an additional 9 months.

3. Rats which received for a period of 12 months diets containing 17.9 mg. arsenic per kilogram in the form of shrimp and of added As_2O_3 showed no evidence of toxicity in their growth, physical appearance and activity or by histological examination of the liver, spleen and kidney.

4. Following the normal process of digestion the arsenic containing substance in shrimp yields a soluble and readily diffusible product which is rapidly eliminated by the kidneys.

5. Arsenic occurs in shrimp in a complex combination and cannot be liberated in the animal organism.

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THE NUTRITIVE VALUE OF ANIMAL TISSUES IN GROWTH, REPRODUCTION AND LACTATION [1]

III. THE NUTRITIVE VALUE OF BEEF HEART, KIDNEY, ROUND AND LIVER AFTER HEATING AND AFTER ALCOHOL EXTRACTION

WALTER H. SEEGERS AND H. A. MATTILL
Biochemical Laboratory, State University of Iowa, Iowa City

FIVE FIGURES

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In 1931 Smith began a series of feeding experiments with liver which were designed to segregate some of its nutritive components. As a first step in this segregation liver was subjected to prolonged extraction with hot alcohol until fat-free, and the residual protein and the extracted materials were examined for their nutritive value in growth and lactation. Such extracted liver fed in a balanced ration no longer supported growth and lactation as does the raw or dried whole tissue (Smith, '31), and relatively large amounts of yeast, cod liver oil or wheat germ oil failed to correct the deficiency (Smith and Seegers, '34). These observations, as well as those of Mapson ('33) pointed to the possibility of hitherto unrecognized accessories readily extractable from liver, whose absence was responsible for the poor nutritive value of rations containing alcohol-extracted liver. In the present work beef heart, round and kidney were similarly studied, and liver in greater detail. A study of the effect of heat on these animal tissues has also been made.

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EXPERIMENTAL PROCEDURE

Preparation of tissues. The tissues were obtained fresh from the slaughter house, trimmed free of adhering fat, ground fine and dried in a Buflovak steam heated drier at 70 to 100°C. The steam pressure was kept below 1 pound per square inch and the drying of 100 pounds of fresh tissue was accomplished in about 4 hours. The tissues were therefore never heated above 100°C. and were subjected to that temperature for only a short time near the end of the drying operation. The products so obtained were the source material for all feeding experiments. The extractor used was of the continuous type briefly described by Sperry ('26).

Animals. In general only male rats were used because of their more rapid growth and uniformity of behavior, and their routine management has been described (Smith and Seegers, '34). Food consumption records were not obtained in all experiments; and only those which seem significant are presented.

Rations. In all the growth studies the protein was fed at a 15 per cent level ($N \times 6.25$). In the nitrogen balance and digestibility studies the proteins of the four animal tissues were fed at a 5 per cent level. In figuring the composition of the rations the non-nitrogenous material of a tissue preparation was arbitrarily considered as carbohydrate. The general composition of all rations used is given in table 1.

GROWTH STUDIES

To determine whether alcohol removed important nutrients from other animal tissues as it does from liver, a comparative study was made with kidney, heart, round and liver extracted with 95 per cent alcohol for 60 hours, and dried in a warm air current. Control animals were fed the respective dried tissues. The composite growth curves are given in figure 1. There was little difference in the growth rate on the diets containing the dried whole tissues. This is in line with the earlier work of McCollum, Simmonds and Parsons ('21) and of Hoagland and Snider ('26). The extracted meats in all

cases gave less satisfactory growth, kidney gave the most rapid, liver the least. It was not surprising to obtain slower growth on the extracted tissues. Rather more striking was the fact that in contrast to kidney, heart and round, the extraction of liver readily removed or destroyed so much of its nutritional value.

TABLE 1

Composition of rations

I. Used in growth studies[1]

a) Alcohol extracted tissues[2]

	Per cent	*Per cent*	*Per cent*	*Per cent*
Liver	18.7			
Round[3]		16.1		
Heart			16.5	
Kidney				16.3
Hydrogenated cottonseed oil[4]	15.0	15.0	15.0	15.0
Corn starch	59.8	62.4	62.0	62.2

b) Dried whole tissues

	Per cent	*Per cent*	*Per cent*	*Per cent*
Liver	23.2			
Round[3]		20.2		
Heart			21.0	
Kidney				21.8
Hydrogenated cottonseed oil[4]	10.7	11.2	10.6	9.7
Corn starch	59.6	62.1	61.9	62.0

II. Used in nitrogen balance and digestibility studies[5]

	Per cent	*Per cent*	*Per cent*	*Per cent*
Liver (dried whole)	7.4			
Extracted liver (130 hours)[6]		6.1		
Heated liver[6]			7.2	
Lard	15.0	15.0	15.0	15.0
Agar agar	2.0	2.0	2.0	2.0
Salt mixture	4.5	4.5	4.5	4.5
Corn starch	71.1	73.5	71.3	78.5
Nitrogen content	0.85	0.84	0.86	0.047

[1] All these rations contained, in addition, agar, 2 per cent; salt mixture (Hawk and Oser, '31), 4.5 per cent, and were supplemented daily with 0.4 cc. cod liver oil and 0.5 gm. yeast (kindly supplied by Northwestern Yeast Company).

[2] The extent of the extraction is stated in the text; 95 per cent alcohol was used.

[3] Included the retail cuts designated as hind shank, round and rump.

[4] Crisco.

[5] Rations containing round, heart, and kidney, were similarly set up to contain 5 per cent of protein.

[6] Supplemented daily with a vitamin B concentrate containing 5.2 mg. N, and with 4 drops of cod liver oil.

A separate comparative study of the nutritive value of beef and pork liver was also made. The raw tissues were dried as described, and the liver ration was modified (table 1) by including 4 per cent yeast and 2 per cent cod liver oil at the expense of an equivalent amount of starch. Fourteen male rats fed beef liver made an average gain of 231 ± 3.3 gm. while thirteen male rats on pork liver gained 223 ± 3.2 gm. during the same 81-day growth period. This slight difference,

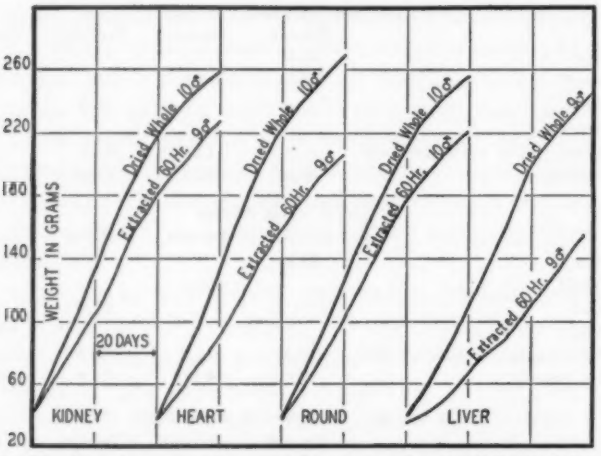

Fig. 1 Showing the composite growth curves of rats fed alcohol-extracted kidney, heart, round and liver. The curves for the respective dried whole tissues are also shown.

in view of the probable errors, hardly demonstrates beef liver to be superior to pork liver for growth purposes. Johnson and Palmer ('34), in a study which had primarily another purpose, fed the raw tissues as small supplements to an otherwise adequate diet and found them to increase growth to an equal extent. From food consumption records the efficiency quotients (Palmer and Kennedy, '31) were computed on the basis of total dry matter consumed (Morris, Palmer and Kennedy, '33). Both liver rations appeared to be equally well utilized. Figure 2 gives the efficiency quotient values for

different segments of the growth curve. It can be seen that during the period of rapid growth different values were obtained for the several segments of the growth curve without change of ration. The quotient tended to increase with advancing age, presumably largely because of increased maintenance costs. This consideration may not justify the application of an efficiency quotient determined on a young animal as a reference for that same animal in subsequent periods of growth. The efficiency index, which requires a standard procedure, appears to be more reliable but is less flexible in its use.

Fig. 2 Illustrating the efficiency quotient values for different segments of the growth curve. The bars represent the segment of the growth curve. The rats weighed from 40 to 50 gm. at the beginning of the experiment.

A preliminary experiment with alcohol-extracted tissues, conducted somewhat differently from the one described above, first emphasized the incipient deleterious effect of moderate high temperatures on nutritive value. After the routine extraction procedure the tissues were dried from 3 to 4 days in an electric oven at 90 to 100°C. This was for the purpose of removing volatile decomposition products which, as judged by odor, largely disappear during the first 24 hours. The rats used were slow growing, being of the same strain and group as those used by Berg ('34) who also considered them

so. Nevertheless the poor showing made by these animals (table 2) seems of real significance. The small amount of fresh round fed to one group increased the growth rate considerably.

These observations suggested that heating for different lengths of time even at temperatures as low as 100°C. might alter the nutritive value. The very slow growth of the animals fed treated liver, as compared with their growth on the other tissues similarly treated, indicated that the nutritive qualities of these meats are not uniformly retained after alcohol extraction and heating. In further studies (fig. 3) the heating

TABLE 2

Average gains made in 50 days on 60-hour alcohol-extracted tissues subsequently dried at 100°C. for 3 to 4 days, and fed at a 15 per cent protein level

Ration	*Male*	*Female*
Liver	70(7)[1]	39(6)
Heart	109(6)	79(4)
Round	102(5)	90(5)
Round + 0.5 gm. raw daily	139(5)	
Kidney	111(4)	80(7)

[1] Number of animals; they weighed from 30 to 40 gm. at the beginning of the experiment.

and extraction times were varied. Liver extracted 1¼ hours[2] was definitely less valuable for growth than dried whole liver. Extracted 60 hours it suffered greatly and large amounts of vitamin G, held by some workers to be especially abundant in liver, did not supply the deficiency. Heating such extracted material for a week at 100°C. further reduced its nutritive value, and another week of heating at that temperature had an additional detrimental effect.

Kidney, which gave the best growth of any of the 60-hour alcohol-extracted tissues, was extracted for 130 hours and heated 2 weeks at 100°C. After this treatment it nevertheless still promoted growth better than liver which had been extracted and heated for only half that time. Unless unknown

[2] This particular extraction procedure was similar to that used by Booher ('33) in preparing vitamin G concentrate from skim milk powder, and it was also used in preparing the vitamin G concentrate used in the present experiments.

accessories are concerned, this difference can be ascribed to differences in the properties of the proteins in these tissues. Since liver is more readily altered it was studied in greater detail.

An attempt was first made to segregate the effects of heat and alcohol. For this purpose the following preparations were fed: a) dried whole liver, b) the same liver heated in an electric oven for 2 weeks at 100°C., c) 130-hour alcohol-extracted liver, and d) liver refluxed with 95 per cent alcohol for 130 hours. The refluxed material was prepared by placing

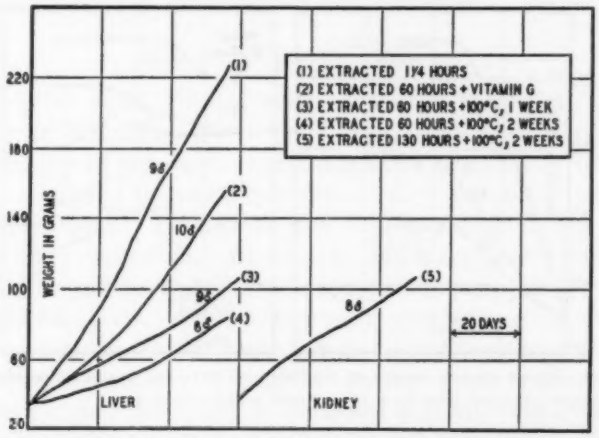

Fig. 3 Shows the effect of varying the hot alcohol extraction time, and the result of heating the residue at 100°C. for 1 and 2 weeks. The greater resistance of kidney is shown.

liver in a large balloon flask kept in a water bath at a temperature slightly above the boiling point of alcohol. After 130 hours the solvent was removed by vacuum distillation and the residue dried in a current of warm air. Exceedingly disagreeable odors (amines?) were given off during the refluxing and these were somewhat noticeable even after the material was incorporated in the basal ration.

The results are summarized in figure 4. Because of the variable behavior of the animals fed 130-hour extracted-liver

some typical individual growth curves are given. A number of deductions can be made from these curves. Heat alone even as low as 100°C., decreased the nutritive value considerably. Boiling alcohol is much more harmful than dry heat at 100°C. showing that hot alcohol acts as a destructive agent in itself, independent of its solvent action. The very poor showing made by the 130-hour extracted material indicates that alcohol may also remove valuable nutrients. While this aspect has been studied further the results are difficult to interpret and are not discussed here. The removal of an

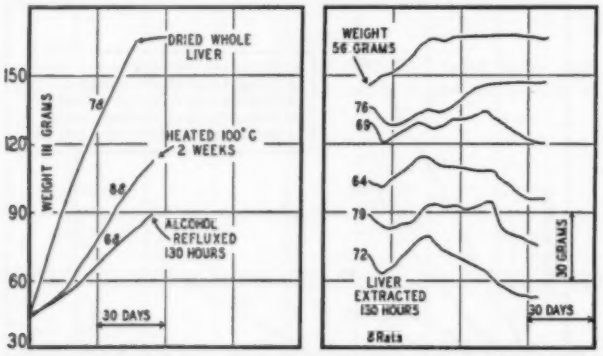

Fig. 4 Shows growth curves of controls and rats fed heated, alcohol-refluxed, and alcohol-extracted liver at a 15 per cent protein level.

unknown factor or factors was indeed suggested by Smith and Seegers ('34) as the primary alteration produced by alcohol extraction, but further information in that direction cannot be obtained until it is certain that the protein component remains unchanged by alcohol extraction.

If protein deficiency is the explanation for the differences between extracted kidney and liver it would follow that their proteins are so organized as to be differently affected by heat and alcohol. Windus, Catherwood and Rose ('31) obtained satisfactory growth on mixtures of highly purified amino acids supplemented with their unknown amino acid, yeast and cod liver oil. Animals on 130-hour alcohol-extracted liver

(15 per cent protein) supplemented with as large or larger amounts of yeast and cod liver oil are in a moribund condition in about 80 days. This comparison suggests that the primary cause of this nutritive failure is in a protein insufficiency. On the assumption that certain amino acids especially lysine and histidine (Greaves and Morgan, '34 a) were made unavailable as the result of alteration in the protein, attempts were made to improve a defective liver ration with ample amounts of lysine and histidine hydrochlorides (0.2 per cent each) but without success. A study of the nitrogen metabolism of animals on these liver diets was next undertaken.

BIOLOGICAL VALUE STUDIES

Biological value studies were conducted according to the method of Mitchell ('24). To insure comparable results it was planned to give each animal the same quantity of food in each period, and the same quantity of nitrogen in the protein feeding periods. This ideal was almost achieved, but difficulties were encountered during the last nitrogen-free period when some of the animals refused to consume all of their portions. In view of the uniformity secured, no correction was made in the computations for metabolic fecal nitrogen as related to food intake. Metabolic urinary nitrogen was based on body weight. Four-day preliminary periods were followed by a 6-day collection period, in one case 5 days.

The products studied were fed in the following order: nitrogen-free, 130-hour extracted liver, dried whole liver, liver heated at 100°C. for 2 weeks, and a final nitrogen-free period. Each animal received daily a yeast concentrate containing 5.2 mg. of nitrogen. In the calculation of the results (table 3) only the second nitrogen-free period was used, because a constant level of nitrogen excretion was just being reached at the end of the first nitrogen-free period, as indicated by the 2-day analyses made. This is a matter of considerable importance (French, '34). The use of the nitrogen-free data from the first period would have given higher and probably less trustworthy figures.

TABLE 3

Average daily metabolic data showing biological value and digestibility of liver preparations

RAT NO.	INITIAL WEIGHT	FINAL WEIGHT	FOOD INTAKE	N INTAKE	FECAL N	ABSORBED N	URINARY N	FOOD N UTILIZED	BIOLOGICAL VALUE	TRUE DIGESTIBILITY[1]
				Nitrogen-free diet						
	gm.	*gm.*	*gm.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*per cent*	*per cent*
1	196	190	9.00	5	10	5	38			
2	212	210	8.00	5	12	5	47			
3	212	203	9.00	5	11	5	46			
4	240	223	6.00	5	9	5	53			
5	196	189	8.00	5	12	5	49			
6	202	195	8.00	5	11	5	37			
7	232	220	7.00	5	9	5	56			
8	238	220	8.00	5	10	5	48			
				Alcohol-extracted liver (130-hour) diet						
1	184	178	6.97	64	48	23	40	5	22	30
2	202	200	8.00	72	44	35	47	12	29	45
3	198	193	9.00	81	66	23	49	5	22	24
4	214	204	6.00	55	40	23	58	6	..	34
5	185	183	8.00	72	54	26	49	1	..	30
6	191	187	6.25	58	47	18	37	4	22	25
7	214	210	7.00	64	47	25	50	0	..	34
8	212	204	8.00	72	45	37	52	12	28	46
									Average	34 ± 1.8
				Dried whole liver diet						
1	176	176	7.00	64	17	54	47	28	52	83
2	204	205	7.98	73	17	63	49	39	62	85
3	192	194	8.98	81	16	73	57	47	60	90
4	200	200	5.98	56	13	50	61	19	38	88
5	181	190	7.98	73	14	66	45	45	68	90
6	184	182	6.00	56	12	51	42	31	61	90
7	210	213	7.98	73	16	65	49	41	63	88
8	202	202	7.98	73	15	67	57	36	54	91
									Average 57 ± 2.1	88 ± 0.6
				Heated liver (100°C., 2 weeks) diet[2]						
1	173	175	6.86	64	22	49	52	19	35	75
2	206	209	7.84	73	22	58	50	33	56	78
3	194	195	8.82	81	26	63	61	33	52	76
4	194	196	5.88	56	18	45	53	21	47	78
5	188	188	5.85	55	16	46	39	31	67	82
6	178	180	5.88	56	18	45	44	22	51	78
7	214	216	7.84	73	18	63	53	36	56	85
8	200	206	7.84	73	23	59	61	24	41	79
									Average 51 ± 2.2	79 ± 0.6
				Nitrogen-free diet						
1	170	158	5.00	5	7	5	20			
2	201	184	4.95	5	7	5	24			
3	188	176	6.76	5	8	5	29			
4	188	182	6.00	5	7	5	27			
5	180	168	5.00	5	7	5	23			
6	172	166	6.00	5	7	5	21			
7	210	205	7.13	5	8	5	24			
8	196	189	8.00	5	9	5	25			

[1] True digestibility = $\frac{\text{Liver N intake—Liver N in feces}}{\text{Liver N intake}} \times 100.$

[2] Five-day collection period.

The biological value of 130-hour alcohol-extracted liver is strikingly low, and similarly the coefficient of digestibility is also low. Apparently after alcohol extraction only a part of the protein is digested yielding a poor assortment of amino acids of relatively small value for metabolic purposes.

The heated material was less strikingly altered, but apparently an analogous change took place. The biological value was only slightly lowered, and hardly enough, in view of the probable error, to account for the pronounced decline in the growth rate of rats fed such heated material (fig. 4). Again, as also with the extracted liver, the digestibility was lowered, but apparently only in proportion to the lowered biological value.

DIGESTIBILITY IN VITRO

In view of the marked changes in digestibility in the animal it seemed desirable to know whether they could be demonstrated by in vitro experiments. Comparative studies on the in vitro digestibility of the other animal tissues after heat and alcohol treatment would also indicate what changes, if any, might be expected in animal experiments.

Procedure. The amount of nitrogen contained in 3 gm. of alcohol-extracted liver (396 mg.) was arbitrarily chosen as the quantity of substrate. The other tissues were adjusted to this on the basis of their nitrogen content. No allowance was made for soluble nitrogen for the reason that the tissues were incorporated in rations on the basis of gross nitrogen content. To the substrate in a 200-cc. beaker was added 50 cc. of a 0.3 per cent Na_2CO_3 solution containing 0.1 per cent NaF, and 10 cc. of a 3 per cent trypsin solution. This was kept at 37°C. for 24 hours. A control, without the enzyme solution, was included. At the end of 24 hours 10 cc. of the enzyme preparation were added to the control and 50 cc. of tungstic acid reagent (Folin-Wu) were added to all the samples which were then centrifuged and filtered. The clear filtrates were diluted to 200 cc. and duplicate Kjeldahl analyses and Sørensen titrations were made on aliquots.

As seen in table 4 the heated meats were less rapidly digested, especially round and liver, than the unheated. Similarly alcohol-extracted liver was not well digested while the same treatment of heart, round and kidney reduced the digestibility only slightly as measured by formol titration and even increased it as measured by the nitrogen in tungstic acid filtrates. The meaning of these contradictory figures has not been studied further. These more or less exploratory experiments on digestion in vitro therefore show marked reductions in digestibility of all the heated meats as compared with the unheated preparation, whereas alcohol extraction has an adverse effect on liver only.

TABLE 4

Digestibility of tissues in vitro, 37°C., 24 hours[1]

TISSUE TREATMENT	FORMOL TITRATION CUBIC CENTIMETERS 0.1 N NAOH				PER CENT NITROGEN NOT PRECIPITATED BY TUNGSTIC ACID			
	Kidney	Heart	Round	Liver	Kidney	Heart	Round	Liver
Dried whole	50.8	45.0	50.0	29.5	46.2	48.5	57.5	31.8
Heated at 120°C., 2 weeks	22.6	10.9	6.7	10.7	35.6	26.5	18.9	12.1
Extracted 130 hours	42.8	40.4	40.0	10.7	74.3	66.6	60.6	15.9

[1] The figures are based on 132 mg. of substrate nitrogen.

DIGESTIBILITY EXPERIMENTS WITH RATS

The effect of heat on proteins especially at higher temperatures has been studied by Morgan ('31), Maynard, Bender and McCay ('32), Maynard and Tunison ('32), Fixsen and Jackson ('32), Schneider ('32), Greaves and Morgan ('34 a, '34 b), Wilgus, Norris and Heuser ('35), and many others. These workers all show that heat, if it is sufficiently intense, impairs the nutritive value of protein.

In view of these many observations and since in the in vitro experiments heat so consistently lowered the digestibility of all the meats it seemed important to verify this by animal

studies particularly with a view to determining whether for a given animal tissue there is a critical temperature beyond which it must be heated before an alteration is produced, and how rapidly alterations take place above that temperature.

These digestibility experiments were conducted in much the same way as described by Adolph and Wu ('34). The thirty-five rats used varied in weight from 60 to 150 gm. The plan was to feed the same amount of the ration daily, for 9 days and collect the excreta during the last 5 days of the period, but with the poorly digestible proteins the animals repeatedly refused their food allotment.[3] To insure approximate figures for true digestibility a protein-free period was also included and no correction was made for the influence of variable food intake on metabolic fecal nitrogen. The following formula was used in computing the digestibility:

$$\frac{\text{N intake}-(\text{fecal N}-\text{N fecal on N-free diet}) \times 100}{\text{N intake}}$$

The general composition of the rations is given in table 1; the heating period was uniformly 3 days and the hot alcohol extraction 130 hours. The results are summarized in figure 5. The digestibility of all these meat proteins was impaired if the heating was sufficiently intense. Kidney was the most resistant and liver the least, while heart and round were between. Even at 110°C. there was some change in liver, but properly fried liver was well digested, presumably because the time of heating is short. Round was still well digested after heating at 120°C. and kidney after 130°C. The effects of heating seem to appear gradually and perhaps they play no important role in the domestic preparation of meats; when the time of heating is short the temperature must be much higher to produce a given effect (compare fig. 3). This

[3] The behavior of these animals was interesting; for example, when round heated at 120°C. for 3 days was fed, they consumed all of their food, but when they were shifted to round heated at 130°C. they promptly refused some of it. In the case of kidney this refusal first occurred when the tissue had been heated at 140°C. The taste of the food, the appetite and tissue requirements doubtless all contributed to this response.

is in general agreement with the conclusions of Scheunert and Bischoff ('30). In contrast to the lowered digestibility of heated meats, that of alcohol extracted tissues was not altered from the normal except in the case of liver (compare table 3).

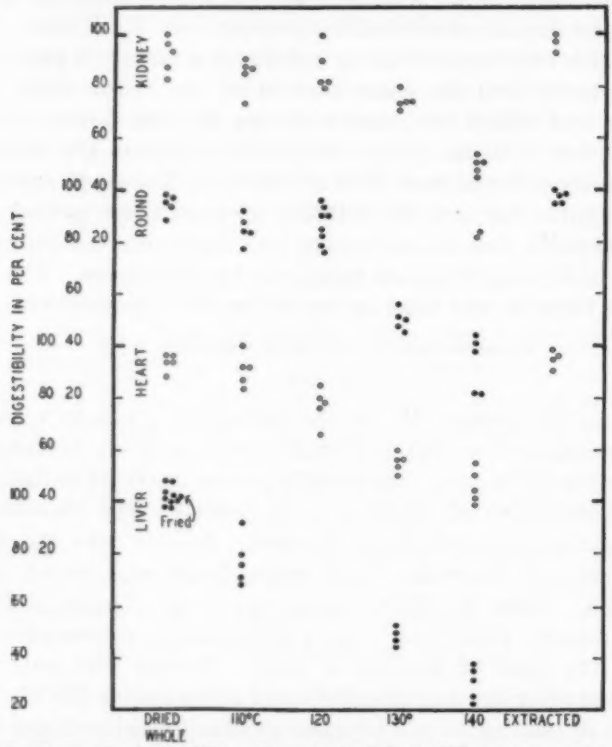

Fig. 5 Shows the digestibility of meats heated at different temperatures for 3 days, or extracted with hot alcohol for 130 hours.

The figures obtained in the in vitro digestion experiments are in rather striking agreement with those obtained on animals.

DISCUSSION

Although it is difficult to distinguish between the effects of heat and alcohol, this series of experiments seems to indi-

cate that alcohol extraction lessens the nutritive value of animal tissues primarily because it removes important dietary accessories, while heating, 1) inactivates such of these as are present in the tissue, and 2) if sufficiently intense or prolonged, modifies the character of the tissue protein in the direction of lowered digestibility.

Recently Wilgus, Morris and Heuser ('35) centered a nutrition investigation of haddock meal on the theory that the lowered growth promoting properties, after heating, are chiefly due to a change in the proteins and also to the destruction of some of the growth promoting activity of the vitamin G complex. While our own data do not in any way indicate which accessories are destroyed, they point to a thermal destruction of growth promoting substances at temperatures where the proteins are not altered. The poor growth on liver heated at 100°C. for 2 weeks (fig. 4) can hardly have been due to the slightly altered protein fed at a 15 per cent level, when the digestibility and biological value of that protein are so little affected (table 3). Had the temperature been slightly higher (110 to 120°C.) such a distinction could not have been made because the digestibility of liver is considerably reduced by such heating. There are no growth data on the other meats after heating. It has been shown, however, that liver proteins are more readily altered by heat than those of other meats, and it is reasonable to assume that their accessories would also be destroyed by heat at temperatures below those required to alter their proteins. When heat is used to destroy accessories in liver tissue (110 to 120°C.) the protein is also made inferior for growth, because it is made indigestible. Prolonged alcohol extraction accomplishes a similar change in liver proteins but not in those of other meats.

Disregarding the vitamin components of a meat, there is limited information which points to yet another cumulative effect, concerned with the alteration of the protein by alcohol extraction and subsequent heating. Thus when kidney was extracted for 130 hours and heated for 2 weeks at 100°C., the biological value was 50 per cent and the digestibility 77 per

cent (corrected), as shown by a limited number of experiments. If alcohol extracted kidney is so completely digestible (fig. 5) and if kidney itself must be heated above 130°C. before its digestibility is lowered one would hardly predict such low figures for alcohol extracted and subsequently heated kidney, especially when kidney is the most resistant to heat. The protein may conceivably be more susceptible to heat alteration after alcohol extraction (compare also table 2). The lowered biological value and digestibility of the alcohol extracted and heated kidney contrasted with the relatively small effect of alcohol alone or heat alone suggests such a possibility. The slow growth rate on this kidney preparation (fig. 3) is thus due to the removal or the destruction by heat (or both) of such accessories as were not adequately supplied by yeast and cod liver oil supplements; and to the lowered nutritive value of the protein.

The inferiority of liver tissue after alcohol-extracting or heating, implies that the protein of liver is peculiarly sensitive to alteration by heat and by hot alcohol, while the other meats are relatively unaffected and are apparently as well utilized after alcohol extraction as before. Their decreased nutritive value after extraction only, may be due solely to the loss of accessories (fig. 1), while liver suffers a double disadvantage in the loss of accessories and in the alteration of the protein by hot alcohol.

The nature of the chemical change induced by heat in animal proteins, especially liver, and by hot alcohol extraction of liver, is suggestive of racemization in that the proteins become refractory to the action of digestive ferments in vitro and in vivo.

SUMMARY

There was little difference in the growth rate of rats fed beef heart, kidney, round and liver at a 15 per cent level of protein in an otherwise apparently complete ration; pork liver was practically equal to beef liver in its capacity to promote growth. When these tissues were extracted in a continuous

extractor for 60 hours with hot 95 per cent alcohol growth was less rapid and was especially slow on extracted liver. Except with liver, whose proteins are altered by such extraction, the difference between the extracted and the unextracted meats can be ascribed solely to the removal of nutrients not adequately supplied by the yeast and cod liver oil supplements.

When the ration contained 130-hour alcohol-extracted liver in place of the 60-hour extracted it did not support growth and the animals died. In contrast to similarly extracted round, heart, and kidney such extracted liver is only slightly digestible and has a low biological value.

Although liver heated at 100°C. for 2 weeks no longer possessed its original capacity to support rapid growth in a balanced ration, its digestibility and the biological value were not lowered appreciably. Above 100°C. there was progressive lowering of the digestibility of the protein as the prolonged heating (3 days) became more intense. Heart and round were resistant to change at 120°C., while kidney was still digestible after heating at 130°C. At higher temperatures these meats also became refractory to digestive enzymes in the animal body as well as in vitro. These changes in digestibility probably play no role in the domestic cooking of meats, but are of importance in the preparation of pure proteins for nutrition studies.

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SOME EFFECTS OF COD LIVER OIL AND WHEAT GERM ON THE RETENTION OF IRON, NITROGEN, PHOSPHORUS, CALCIUM AND MAGNESIUM DURING HUMAN PREGNANCY[1]

CALLIE MAE COONS AND R. R. COONS

Department of Agricultural Chemistry Research, Oklahoma Agricultural and Mechanical College, Stillwater

ONE FIGURE

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The use of continuous controlled experiments to measure the effects of specific dietary factors on the retention of minerals during human pregnancy has been seldom employed. Numerous difficulties concur to hinder these studies from being carried out over long periods of time with the subject restricted to a constant diet and routine of living habits. The time has come, however, in our stage of information when such procedures can be applied safely and ethically with corresponding gains in the type and quality of data obtained.

The results reported in this paper covered 101 days of continuous regulated diet during the last half of pregnancy. During 82 of the days the diet was weighed, while 19 days represented an intermission for possible recovery from therapy during which period the diet remained almost the same but no weighings or collections were made. Analyses are presented for eighteen metabolic periods of 4 days each during which balances of nitrogen, iron, phosphorus, calcium and magnesium were determined and records of creatinine

[1] Published with the permission of the director of the Oklahoma Agricultural Experiment Station.

elimination, urine volume, pH and specific gravity obtained.

Two questions which, for reasons to be discussed below, seemed eminently in need of investigation under these controlled experimental conditions concerned the quantitative effect of cod liver oil on calcium and phosphorus metabolism and the influence of some part of the vitamin B complex on nitrogen and iron retention during pregnancy. An effort was made to study the two simultaneously.

LITERATURE

In most of the studies in metabolism during human pregnancy reported in the literature the diet has been self-chosen and more or less variable from day to day. One case described by Macy and co-workers ('31) received a specified diet for 21 days divided into two non-continuous periods. Examples of prolonged studies with subjects receiving the varied home diets are those of Wilson ('16), two cases for 19 and 15 weeks each, and Hoffström's one case for 23 weeks ('10).

The benefits of cod liver oil on mineral metabolism in adults has not been demonstrated as conclusively as with children, despite its widespread use in pregnancy. Improvements noted usually have been slight. The women of Toverud's group ('31) who received cod liver oil showed some increases in retention of calcium but responded more favorably to higher mineral intakes. Two of the subjects previously reported by the writer (Coons and Blunt, '30; Coons et al., '34) were receiving cod liver oil routinely but showed no marked advantages over others of the groups in the retention of calcium and phosphorus. For clinical purposes, therefore, it has seemed worthwhile to study the effect on mineral retention during gestation when cod liver oil was added to a diet reasonably adequate in calcium and phosphorus. Furthermore, in the light of the decidedly superior retention of these two elements shown by women receiving reasonable amounts of potent sunshine (Coons et al., '35), it was desirable to measure the response to cod liver oil therapy under

more controlled conditions to know to what extent it could substitute for sunlight in its effect on calcium and phosphorus metabolism during gestation.

Interest in the factors affecting iron and nitrogen retention has been indicated in previous reports from this laboratory. Iron balances with a group of Chicago women showed fair retentions as compared to fetal needs (Coons, '32), while those with a group of Oklahoma women receiving even better iron intakes indicated poor utilization of iron simultaneous with excellent retentions of calcium (Coons et al., '35). Concentrates of vitamin B have shown a supplementary effect on the diets of college women of this section (Coons et al., '31). Since whole grain cereals were lacking in both sets of diets (Coons, '33), and since individual exceptions in the pregnancy group showed best iron retention, it was suggested that the deficiency might be some component of, or a factor associated with, the vitamin B complex. Reputed values of whole grain cereals in hemoglobin regeneration supported this view (Rose and Vahlteich, '32). Harris ('34) commenting recently on various reports of the relationship of vitamin B to anemia has referred to "the effect of a vitamin B deficiency in bringing about some error in iron metabolism of a nature not yet clearly understood."

Some factor associated with vitamin G and responsible for remission in certain anemias is being widely investigated at present. Varying degrees of success have been reported for marmite (Wills, '33), rice polishings (Miller and Rhoads, '34), yeast and wheat embryo (Ungley and James, '34), when used as substitutes for liver therapy in various anemias. Reports of studies concerning the effect of any vitamin B preparation on the utilization of dietary iron during a period of normally increased iron demand seem, therefore, timely at least.

EXPERIMENTAL STUDIES

The subject. The primipara used for this study was experienced as a subject for constant diet regimen as well as balance experiment procedures. Prior to the beginning of

pregnancy she had served 28 days on self-chosen diets with collections of excreta and at another time 36 days in a similar experiment requiring a restricted constant diet as well as collections (Reder and Coons).

The pregravid studies had shown that the subject could remain in slight negative balance on calcium intakes of 0.30 to 0.35 gm. daily and fluctuated from positive to negative on intakes of 1.0 gm. or over. Weight records scattered over the previous 8 years showed less than 5 pounds maximum variation from the 142 which existed at the beginning of this pregnancy and which was only 2 pounds below the average for her height and age. Also there had been no loss of time from work due to illness over a period of 10 years prior to this experiment. These facts indicated a fairly stable condition of health and reasonably adequate dietary habits and therefore a subject who could be regarded as 'normal' and with average nutritional reserves for the metabolism studies which were planned.

The pregnancy was uneventful except for a slight rise in blood pressure which developed during the last 2 weeks antepartum and which reached a maximum of 148/80 mm., but this was 4 weeks after the termination of the metabolism work. Gestation lasted 293 days, 42 weeks, after the beginning of the last menstrual period. The infant weighed 9 pounds, 9 ounces.

Metabolic studies including control and preliminary periods extended from the twenty-first to the thirty-sixth weeks, covering the seasons from mid-January to the last of April. In order to measure the possible effect of cod liver oil it was necessary that the subject avoid sunshine throughout all control and supplemented periods. This was accomplished by work indoors during daylight hours and in a north room most of the time. Similar precautions for protection from light were taken when conveyance was necessary in daytime. All activities were reduced to a regular daily schedule.

The diet. The diet planned was one as well suited to the taste preferences of the subject as experimental conditions

would allow. Meat was omitted from the first, lest later threatening signs of toxemia should necessitate radical changes in the basic diet or termination of the experiment entirely. Weight gains during the first half of the study justified a reduction in the amounts of certain high calorie foods for the last half. The diet was planned to contain about 1.2 gm. of calcium daily in addition to fair amounts of other minerals because this quantity of calcium seemed nearly adequate for women living under the influence of the sunlight of this region.

For the first half of the study, series A, the basic diet consisted daily of the following items with quantities stated in grams.

Breakfast: orange juice, strained, 200; grapenuts cereal, 50; milk, 300.
Lunch: Iceberg lettuce, 100; mayonnaise, 20; butter, 50; whole wheat bread, 150; tomato juice, canned, 200; peaches, canned halves, 200; graham crackers, 50; eggs, 100.
Supper: milk, 400; potatoes, boiled in jackets and peeled, 200; peas, canned, 100; peaches, canned halves, 200; graham crackers, 50.
Distilled water, ad libitum. No salt in cooking or serving.

The items were identical for the second half, series B, but with reductions in certain quantities:

Whole wheat bread, reduced from 150 to 100 gm. daily
Butter, from 50 to 30 gm.
Potatoes, from 200 to 100 gm.
Eggs, from 100 to 50 gm.

A wheat germ preparation,[2] 30 gm. daily, or the ash of 30 gm., supplemented the above diet in certain periods and in others 15 gm. of standard cod liver oil[3] were supplied daily. The ash of the wheat embryo preparation was used alone in one period of each series because it was impossible to find a concentrate which did not change appreciably the mineral content of the diet and thereby prevent definite conclusions as to the supplementary effect from the vitamins alone.

The source, variety and brand of all foods used remained the same throughout both series. Eggs came from the same

[2] Squibb's Vitavose.
[3] Mead's Standardized Cod Liver Oil, purchased in open market.

flock of pure bred hens, bread from the same bakery and the same oven output each day, and potatoes were bought in two lots, one for each series. Canned foods, such as peaches, peas and tomato juice were purchased in case lots, but each can was not opened until needed for use, and the contents were drained of liquids before being weighed. The quality of lettuce varied greatly because of the market season covered by the experiment. The same may have been true for eggs, since the experiment included both early and late laying periods. Doubtless the composition of the bread varied much from time to time, as did also the iron content of foods from cans, particularly tomato juice. In spite of strict precautions the composition of the analyzed diets showed considerable variation and emphasizes the necessity for actual analysis of each diet as the only accurate procedure for balance experiments.

Collections. Each experimental series consisted of nine 4-day collection periods preceded by a 4-day preliminary period and followed by a 1-day lag period, throughout which the weighed diet remained the same. The methods of collections and analysis were identical with those regularly employed in this laboratory (Coons, '30; Coons et al., '35). An aliquot of each item of the diet was weighed into a composite which was analyzed for each element. This procedure seemed more practical and accurate for a prolonged series than did the preparation and preservation of each dietary item in homogeneous mixtures and quantities to last throughout the study. Supplements, when used, were added to the composite for analysis in the same aliquots as were other ingredients.

Urine was not analyzed for iron as for all other elements because previous studies had demonstrated that the amount excreted by way of urine was too small to affect the final balance appreciably and that the urinary excretion of iron was surprisingly constant from period to period for each individual (Coons, '32; Coons et al., '35).

RESULTS AND DISCUSSION

The intake and outgo balances for iron, nitrogen, phosphorus, calcium and magnesium are shown in tables 1 and 2, while the graph in figure 1 demonstrates the interrelation between the curves of storage for these elements.

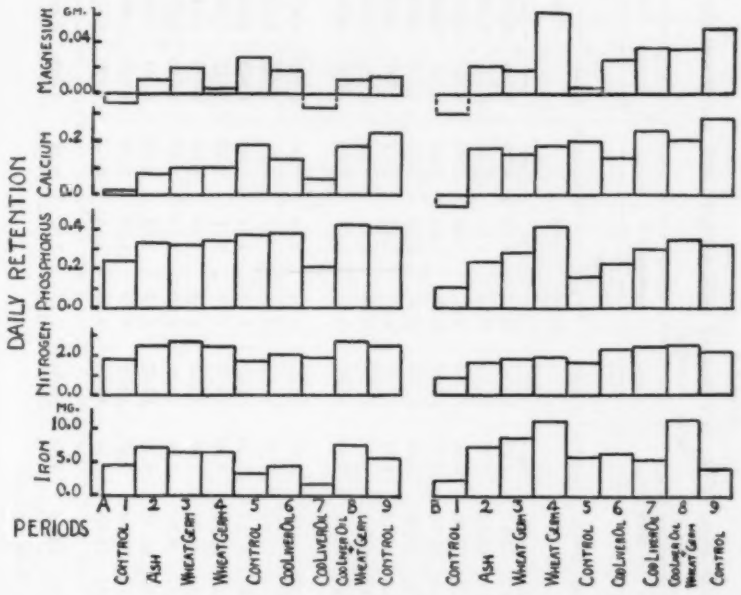

Fig. 1 The effect of various supplements to the basic diet on the rates of retention of iron, nitrogen, phosphorus, calcium and magnesium during the last half of pregnancy.

Iron. Both intake and storage of iron were good. The basic diet supplied approximately 18 to 20 mg. of iron daily, with 6 to 8 gm. added through the wheat germ preparation in four periods of each series. In all periods the intake was much above the 15 mg. recommended for adult dietaries (Sherman, '32) and approximated the 20 mg. suggested as tentative standards for pregnancy (Macy and Hunscher, '34).

TABLE 1

The effect of cod liver oil and wheat germ on the retention of iron, nitrogen and phosphorus during pregnancy (daily averages)

SERIES AND PERIOD	SUPPLEMENT TO BASIC DIET	WEEK [1] OF PREGNANCY	IRON			NITROGEN				PHOSPHORUS			
			Food	Feces	Balance	Intake	Outgo		Balance	Intake	Outgo		Balance
							Urine	Feces			Urine	Feces	
			mg.	*mg.*	*mg.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
A 1	None, control	22	19.64	14.97	4.67	12.94	9.25	1.40	1.81	1.988	0.755	0.998	0.235
A 2	Wheat germ ash	23	27.04	19.94	7.10	13.19	9.24	1.42	2.53	2.333	0.930	1.066	0.337
A 3	Wheat germ	23	27.28	20.48	6.80	13.24	9.02	1.54	2.68	2.254	0.870	1.057	0.327
A 4	Wheat germ	24	26.03	19.22	6.81	12.94	8.82	1.68	2.44	2.237	0.810	1.085	0.342
A 5	None, control	24	20.13	16.68	3.45	12.19	9.10	1.39	1.70	2.043	0.784	0.884	0.375
A 6	Cod liver oil	25	21.43	16.92	4.51	12.21	8.62	1.42	2.17	2.032	0.749	0.900	0.383
A 7	Cod liver oil	25	22.42	20.46	1.96	12.25	8.66	1.64	1.95	2.024	0.812	0.999	0.213
A 8	Wheat germ and cod liver oil	26	28.03	20.14	7.89	12.97	8.64	1.66	2.67	2.299	0.888	0.984	0.427
A 9	None, control	27	21.02	15.38	5.64	13.25	9.40	1.39	2.46	2.115	0.878	0.826	0.411
B 1	None, control	31	18.88	16.61	2.27	10.19	8.01	1.27	0.91	1.768	0.736	0.920	0.112
B 2	Wheat germ ash	31	28.31	21.11	7.20	10.60	7.71	1.24	1.65	1.954	0.819	0.899	0.236
B 3	Wheat germ	32	32.14	23.34	8.90	10.76	7.59	1.31	1.86	1.945	0.792	0.870	0.283
B 4	Wheat germ	32	27.35	16.26	11.09	10.45	7.18	1.29	1.98	1.927	0.745	0.772	0.410
B 5	None, control	33	22.27	16.33	5.94	10.25	7.30	1.31	1.65	1.630	0.730	0.736	0.164
B 6	Cod liver oil	34	18.70	12.33	6.37	10.62	7.00	1.23	2.39	1.644	0.664	0.750	0.230
B 7	Cod liver oil	34	18.72	12.98	5.24	10.29	6.55	1.25	2.50	1.617	0.621	0.696	0.300
B 8	Wheat germ and cod liver oil	35	23.94	12.63	11.31	10.76	6.80	1.41	2.55	1.899	0.732	0.822	0.345
B 9	None, control	35	17.94	13.86	4.08	10.61	7.17	1.21	2.24	1.668	0.698	0.645	0.325

[1] The week which included most of the 4-day period.

TABLE 2

The effect of cod liver oil and wheat germ on the retention of calcium and magnesium during pregnancy (daily averages)

SERIES AND PERIOD	SUPPLEMENT TO BASIC DIET	WEEK [1] OF PREGNANCY	CALCIUM				MAGNESIUM			
			Intake	Outgo		Balance	Intake	Outgo		Balance
				Urine	Feces			Urine	Feces	
			gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
A 1	None, control	22	1.265	0.140	1.101	0.024	0.424	0.119	0.312	—0.007
A 2	Wheat germ ash	23	1.326	0.141	1.106	0.079	0.554	0.126	0.417	0.011
A 3	Wheat germ	23	1.318	0.136	1.081	0.101	0.561	0.121	0.420	0.020
A 4	Wheat germ	24	1.314	0.132	1.081	0.101	0.551	0.118	0.428	0.005
A 5	None, control	24	1.310	0.123	1.000	0.187	0.441	0.115	0.298	0.028
A 6	Cod liver oil	25	1.277	0.145	0.991	0.141	0.443	0.114	0.311	0.018
A 7	Cod liver oil	25	1.285	0.163	1.057	0.065	0.453	0.097	0.366	—0.010
A 8	Wheat germ and cod liver oil	26	1.330	0.177	0.971	0.182	0.551	0.105	0.435	0.011
A 9	None, control	27	1.281	0.176	0.874	0.231	0.448	0.115	0.320	0.013
B 1	None, control	31	1.191	0.127	1.101	—0.037	0.377	0.117	0.275	—0.015
B 2	Wheat germ ash	31	1.215	0.132	1.008	0.175	0.482	0.124	0.337	0.021
B 3	Wheat germ	32	1.184	0.112	0.922	0.150	0.475	0.110	0.347	0.018
B 4	Wheat germ	32	1.198	0.160	0.857	0.181	0.468	0.109	0.296	0.063
B 5	None, control	33	1.174	0.102	0.871	0.201	0.369	0.118	0.245	0.006
B 6	Cod liver oil	34	1.153	0.119	0.894	0.140	0.377	0.098	0.253	0.026
B 7	Cod liver oil	34	1.186	0.120	0.822	0.244	0.379	0.088	0.256	0.035
B 8	Wheat germ and cod liver oil	35	1.205	0.098	0.894	0.213	0.474	0.079	0.361	0.034
B 9	None, control	35	1.149	0.095	0.775	0.279	0.371	0.072	0.248	0.051

[1] The week which included most of the 4-day period.

The storage ranged from nearly 2 mg. daily in one of the periods with added cod liver oil to more than 11 mg. daily in two different periods with added wheat germ. The highest retention represented 45 per cent of the intake while the average for the eighteen periods was 26 per cent of the amount ingested. The response to the addition of wheat germ was immediate in all instances.

Periods A 2 and B 2 show clearly, however, that most of the improvement in storage was due to the inorganic portion of the wheat germ represented by the ash. Rose and coworkers ('32, '34) have reported similar results from the ash and the organic portions of whole wheat and certain other cereals when measured by hemoglobin regeneration in the nutritional anemia of the rat, except that they associated the benefit with the bran portion of the whole grain. Doubtless some of the supplemental value of the ash was due to a greater supply of available iron, perhaps some to the supply of needed copper or other elements (Schultze et al., '34; Sherman, Elvehjem and Hart, '34).

The progressively greater storage in periods B 3 and B 4 indicated some benefit from the organic portion of the wheat germ, but corresponding periods in series A do not support this view. In periods A 8 and B 8, doubly supplemented, the improvement in storage represented approximately 100 per cent of the increase in iron intake, hence optimum utilization of the added iron. Another fact indicating that the supplementary effect of the wheat germ was due to more than its iron content was the high storage of period B 8 with wheat germ as contrasted to that of B 5, a control with the same iron intake, and the accomplishment of this retention through the reduction of fecal iron.

In either series the wheat germ in conjunction with the cod liver oil gave the best retention found. As will be shown later the same was true for nitrogen. Cod liver oil alone seemed to be without effect on the iron retention under the conditions of this experiment (periods A 7 and B 7).

Storage tended to be lower in series A than in series B in spite of reductions in intake in the latter. The daily averages were 5.42 and 6.93 mg. of iron, respectively. The advance of pregnancy may have been a factor in stimulating to greater iron utilization in the last series. On the other hand, the omission of half of the eggs in this series may have improved the retention by providing fewer sulfide radicals to divert both copper and iron from absorption according to the mechanism described by Sherman and co-workers ('34).

On the whole the storage of iron by this individual was much above that previously found for other gravid subjects. The average for this woman equalled the maximum previously recorded. For this woman the average intake during all periods was 23.37 mg. daily, as compared to 14.72 (Coons, '32) and 15.74 mg. (Coons et al., '35) for other groups, while the average retention of 6.18 by this case corresponded to 3.16 and 2.45 mg. daily for the other studies.

Some of the high quality of the diet might be attributed to the large quantity of peaches, 400 gm. daily, along with generous amounts of whole wheat, since the former have been found to be so potent for blood regeneration (Robscheit-Robbins, '33). While these items may have contributed to the good storage found on the basic diet, the experiment showed that such storage could be further augmented by the wheat germ preparation.

The average daily fecal iron tended to remain constant (Coons, '32) from period to period except as influenced by marked changes in intake and these did not always alter the fecal output of iron. The iron content of dried feces varied widely, from 0.4196 to 0.8076 mg. per gram of dried stool, but was more consistent than found in this laboratory for different individuals on home diets for whom the range was five-fold, 0.2748 to 1.2840 mg. of iron per gram.

Some light on the question of iron utilization is gained from the similar curves for iron and nitrogen shown in figure 1. Elsewhere (Coons et al., '35) poor iron storage has been associated with deficient nitrogen economy. This is to

be expected wherever formation of whole blood is dependent upon exogenous supplies of both iron and nitrogen.

If the rate of iron retention accomplished by this subject throughout most of the last half of pregnancy is a desirable standard, then many of the balances reported heretofore were deficient and the estimated fetal needs of 4.75 mg. daily in the last trimester of gestation (White House Conference, '32) represents by no means the total needs of gravidity. The need for more data from controlled metabolic studies is obvious in view of the prevalence of anemias of dietary and gastro-intestinal origin particularly in pregnancy and infancy. One must keep in mind the fact that iron retention is only one step in the direction of iron utilization and of subsequent whole blood formation.

Nitrogen. Nitrogen retention was quite uniform throughout the experiment. The storage amounted to approximately 2 gm. of nitrogen daily in seventeen of the eighteen metabolic periods with an average of 2.15 gm. for all of both series. This was in accord with an observation previously noted that published data on nitrogen balances during pregnancy showed a definite tendency to a common storage around 2 gm. daily (Coons and Marshall, '34; Macy and Hunscher, '34). The influence of the advance of pregnancy was not enough to overcome the 20 per cent reduction in intake and maintain a level of storage in series B equivalent to that found in series A. The daily averages for the two were 1.97 and 2.27 gm., respectively.

Lowest retention occurred in the control periods, each supplement being associated with better storage, the wheat germ slightly more. Best retentions accompanied the use of both supplements simultaneously, but the differences were not marked. The better nitrogen storage observed during the periods with only the ash of the wheat germ added to the basic diet was related, doubtless, to the improved inorganic salt metabolism as manifested by better storage for all minerals studied (fig. 1, periods A 2 and B 2).

There was no evidence from these data that the benefits from the supplements were by way of increased absorption of nitrogen. Fecal nitrogen showed a utilization of 87 to 90 per cent of the nitrogen intake from period to period. This seemed to be a normal rate for such individuals (Coons et al., '35).

Probably the experimental diet, as planned, was less adequate in protein than in any other food essential. The nitrogen intake (table 1) was 12 to 13 gm. during series A and approximately 10.5 gm. during series B. It varied less than that of any other element studied in this supposedly constant diet, due, doubtless, to the fact that most of the nitrogen came from only two sources, milk and eggs, which tend to be more uniform in structure and nitrogen content than do other protein-containing foods such as meats.

Figure 1 shows that in period A 7, with the cod liver oil addition to the basic diet, the lowest storage in the series was found for every element studied. The nitrogen coefficient of digestibility was 86.6 per cent, which was as low as any found for the entire experiment. Evidence from original data indicated that although the number of stools was regular for this period, the percentage of moisture was higher and the average total dry feces per day was the largest found in any period of either series. The cause for this increased fecal output was not clear. However, as will be seen later, a higher urinary excretion also contributed to the lower balances recorded for calcium, phosphorus and magnesium, suggesting thereby some error in metabolism in general, not alone in absorption.

Phosphorus. Fluctuations in retention of phosphorus paralleled those for nitrogen when they differed from calcium (fig. 1). Supplements tended to improve the storage only slightly.

Retentions were better in series A with the higher intake than in series B. The daily averages were 0.338 and 0.267 gm., respectively, from corresponding average intakes of 2.147 and 1.783 gm. of phosphorus. The storage rates in periods B 1

and B 5, control periods on the lower diet, were inadequate for the total needs of gestation at that time. However, at the lower level of intake, 1.6 gm. daily, cod liver oil was more effective in improving the retentions than at intake levels of 2.0 gm. or over. As in previous studies the storage rates for phosphorus indicated that the total gestational needs for this element are more uniform throughout pregnancy than the requirement of calcium, magnesium or iron (Coons et al., '35).

In each series the control periods, A 9 and B 9, which followed the three consecutive cod liver oil periods, supported a rate of phosphorus storage equivalent in quantity to that in the preceding supplemented periods and that in spite of reductions in phosphorus intake. These findings suggest, among other things, a delayed effect of the cod liver oil which was not maximum even after 12 days of administration of 15 gm. daily. Such a retarded response may mean that the dosage was insufficient for prompt action or that the benefit to phosphorus utilization was secondary to the effect on calcium metabolism. The data for calcium retention, however, do not support the latter view.

The utilization of dietary phosphorus under the conditions of this experiment was better than was found for a group of Chicago women who had an average storage of 0.161 gm. on an intake of 1.431 gm. of phosphorus daily. However, the cod liver oil therapy was required to promote a phosphorus utilization equal to that shown by Oklahoma women under the influence of sunlight and with the same dietary level (periods B 6 to B 9). The latter had an average storage of 0.299 gm. daily on intakes of 1.632 gm. of phosphorus (Coons et al., '35) as compared to an average intake of 1.965 gm. and a storage of 0.302 gm. daily for the entire eighteen periods of this study. Data from these two investigations suggest that intakes of phosphorus as low as 1.6 gm. daily approach the minimum level of adequacy, even with reinforcement from cod liver oil or sunshine. Intakes around 2.0 gm. daily gave uniformly better retentions under the variety of conditions which prevailed in the two studies.

Calcium. Calcium balances, like those for iron, constituted one of the high points of interest in the original plans for this experiment. Final analysis revealed a storage much below the expected rate and with one negative balance.

The calcium intake (table 2) was only a little over 1 gm. of calcium daily and was 0.10 gm. less in the second series than in the first. At the intake level used and as planned poor storage had been demonstrated by women without access to potent sunshine (Coons and Blunt, '30) and with or without varying doses of cod liver oil added to self-chosen diets. On the other hand, at this same intake level good storage had been recorded for women of this section receiving a reasonable amount of sunlight (Coons et al., '35).

In the last group intakes of 1.2 gm. or less daily resulted repeatedly in retentions above 0.30 gm. of calcium daily. In the eighteen balance periods of the present study the retention did not at any time reach this figure, which represented the maximum daily deposit deemed necessary for the fetus alone. The diet for this subject possessed almost every advantage, except for calcium content, over the self-chosen diets of both groups previously studied.

The response to cod liver oil administration was slight when the storage of calcium was compared from period to period or with preceding control periods. On the other hand, in each series the highest calcium retention, like that of phosphorus, occurred in the control period following the cod liver oil therapy. Whether this represented a restorative after-effect of the therapy or was evidence of a delayed action of the supplement or of the cumulative effect of a subadequate dosage remains to be investigated. The urinary calcium remained constant for the last two periods of each series and reductions in fecal calcium provided the augmented retentions which marked the final control periods.

During the consecutive final months of gestation touched by the balances in this experiment the estimated daily fetal need for calcium ranged as follows: 0.055, 0.137, 0.225 and 0.306 gm. (Coons et al., '35). The corresponding average

storage rates by months for this subject were: 0.098, 0.155, 0.136 and 0.215 gm. daily. The 2 months with highest averages each included the 12 days of cod liver oil administration. For all eighteen periods the average storage was 0.152 gm. of calcium daily. Obviously the quantities retained were scarcely sufficient for the needs of the fetus alone.

Additional evidence of inadequate retention of calcium was that the level of storage in the second series was higher than in the first in spite of slight reductions in diet, and further, that there was a gradual increase in the rate of retention during each series, particularly the last. The highest storage found was in the final control period and on the lowest intake. Such increasing calcium storage coincident with the advance of late gestation has been interpreted in previous studies as indicating subadequate retention and failure to replenish maternal reserves prior to the heavy fetal demands of this period.

The tentative conclusion seems warranted, therefore, that under the conditions of this experiment 15 gm. of cod liver oil daily was not equivalent in its effect on calcium retention during human pregnancy to that of incidental but reasonable amounts of irradiation with the sunlight of this section during the spring and fall months. The need for similar observations on other individuals is obvious.

No effect of wheat germ on calcium metabolism was demonstrated by this experiment. During periods characterized by the wheat germ supplement alone retention fluctuated and was similar to that during the period containing the ash of wheat germ. On the other hand, it was not clear whether the better storage of periods A 8 and B 8 was due to cod liver oil alone or to a summed effect of both supplements, as for example through the improved phosphorus content of the diet.

An optimum acid-base balance of the diet for pregnancy has been described with reference to calcium retention (Coons et al., '34). Although the acid-base balance of this diet was not determined, the urine pH during this experiment ranged

close to that previously found associated with good calcium utilization (Coons et al., '35). Thus in series A the pH was 7.2 in all periods except A 2 when it was 7.4, and A 3 and A 4, when it fell to 7.0. Correspondingly in series B it was 7.1 to 7.2, except for 7.3 during the feeding of the ash of wheat germ, and 6.9 during the two periods with 30 gm. daily of unashed wheat germ.

Figure 1 shows that the curve for calcium storage was erratic and widely fluctuating, comparing in this behavior to that of iron and of magnesium. Retention varied ten-fold between the highest and lowest figures and as much as 100 per cent in consecutive periods with the same diet treatment. The curve for calcium retention was not related to that of any other mineral although it most resembled that for magnesium.

Urinary calcium, although fairly constant from period to period, was less in the second than in the first series, and constituted 10 to 12 per cent of the total output. Marked changes in storage were accomplished mainly through reductions in fecal calcium.

The calcium data of this experiment indicate the need for controlled studies of the effect of larger doses of cod liver oil, of a comparison between the effect of cod liver oil and potent sunshine on the same individual, and of the action of other sources of vitamin D, irradiated ergosterol and ultraviolet irradiation of the individual, on calcium and phosphorus metabolism to determine whether the benefits from these factors are similar to those derived from moderate uses of potent sunlight. The increasing data on the multiple nature of vitamin D are significant in this connection.

Magnesium. In spite of an apparently adequate intake of magnesium (table 2) three negative balances occurred, two others amounted to equilibrium and all were poor. An average daily intake of 0.455 gm., range 0.369 to 0.561, gave a retention of 0.018 gm. daily. This was a lower utilization than was found for the women receiving Oklahoma sunlight and storing an average of 0.063 gm. from an intake of 0.394

gm. daily. Cod liver oil improved the retention only slightly in series B. As with calcium, a higher storage was found immediately following the cod liver oil administration (period B 9). Increases in magnesium intake, as from wheat germ, also improved the storage only slightly and irregularly. Some influence of the progress of gestation on the demands for magnesium was indicated by the average storage for series A of 0.009 gm. daily and for series B of 0.027 gm., corresponding to intakes of 0.492 and 0.419 gm. daily.

Figure 1 also shows the relation of the curve of magnesium retention to that of calcium. In periods A 3, 5 and 9, also in periods B 3, 8 and 9, the calcium and magnesium curves vary in the same direction and in opposition to those of one or more other elements. They were diverse to each other only in periods B 5 and B 6, although the proportions in B 4 resemble those for phosphorus and iron more than for calcium.

Here again is evidence that magnesium metabolism is not antagonistic to that of calcium when the intake amounts to no more than is found ordinarily in dietaries (Coons et al., '35). The existence of common factors governing the utilization of calcium and magnesium had been assumed from previously reported instances of poor magnesium retentions associated with poor calcium storage, or in other cases parallel good retention of the two elements.

Urinary magnesium was fairly constant when compared to variations in intake but fluctuated with increases or decreases in retention. On the other hand, fecal magnesium rose with the intake augmented from wheat germ and fell with reversion to the basic diet. For these reasons urinary magnesium varied from 10 to 35 per cent of the total output of this element.

General discussion. The foregoing data show that even during a condition of fairly constant normal physiological demands, such as growth, and under the regimen of a presumably constant diet, the amounts of different elements retained varied from week to week. Calcium, iron and mag-

nesium retentions fluctuated more than did phosphorus and the last more than nitrogen. Similar variations in mineral metabolism had been demonstrated already by this laboratory for non-gravid women on a regulated diet (Reder and Coons). Under the conditions of this experiment such irregular metabolic behavior is not easily explained by variations in demand or supply of these elements.

Reference has been made to the varied composition of this supposedly constant diet. Some of the marked fluctuations would have seemed incredible after the care known to have been used in preparing and weighing the diets, had not earlier experiences predicted such. Attention is called to instances in which changes in output corroborated the analyses of the diets (compare iron, period B 8; phosphorus, period B 1). Marked increases aside from those due to the inorganic portion of the wheat germ were most frequent in the case of iron. Some of this was due to the ease of contamination of food with this element during preparation for the table. Part of it has been explained by the acid canned foods used. Table 1 shows that the peak of irregularity centered around period B 3, tapering away by the end of period B 5, hence probably represented simultaneous contamination from more than one food source. The phosphorus content of the diet varied next most, due probably to differences in the baker's mixes of whole wheat bread. Dietary calcium was less constant than was magnesium, while nitrogen was most constant of all. Usually the variations, even of nitrogen, were sufficient to have masked true differences in retention if composites for each day had not been analyzed completely. In common with the findings of MacKay and Butler ('35) and of Toscani and Reznikoff ('34) these data show that the analysis of samples of every food item from each experimental diet and period is an indispensable routine in the determination of biochemical balances.

SUMMARY

The practicability of maintaining a human subject on a prescribed diet, constant in quality and quantity, for experimental purposes covering eighteen metabolic periods and 101 consecutive days of pregnancy has been demonstrated.

Cod liver oil and a wheat germ preparation were used to supplement a basic diet in order to study the effect on the retentions of calcium, phosphorus, magnesium, iron and nitrogen under strict control of activities and environmental conditions.

The basic diet supported good retention of iron, but excellent storage was accomplished with the aid of the wheat germ preparation. The results were traced largely to the ash of the preparation rather than to the organic portion. The latter exerted a slight influence. Nitrogen retention was also improved by this supplement. Cod liver oil further supplemented the wheat germ in its effect on nitrogen and iron retention.

Cod liver oil to the extent of 15 gm. daily improved the retention of calcium, phosphorus and magnesium only slightly and irregularly. It did not raise the calcium and magnesium storage to the level previously recorded for a group of gravid women who had access to reasonable amounts of the sunshine of Oklahoma. The rate of calcium retention did not cover the fetal demands for the time involved.

Evidence of the influence of the progress of gestation on the rates of retention of the elements studied was strongest for calcium, iron and magnesium. The curves of retention for nitrogen most resembled that for iron, while calcium and magnesium of the minerals were most consistently related.

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THE FOOD VALUE OF ETHYL ALCOHOL

H. H. MITCHELL
Division of Animal Nutrition, University of Illinois, Urbana

(Received for publication May 25, 1935)

Although the ability of the animal body to absorb and to oxidize ingested ethyl alcohol was early established, the physiological value of the energy thus liberated is still uncertain, in spite of numerous experimental contributions to the subject. Space will not permit a comprehensive review of this voluminous literature; it must suffice to refer to some of the most recent work, and only as it bears on the problem studied in the investigation to be reported here.

The utmost confusion prevails in regard to the question whether the energy liberated by the oxidation of alcohol is necessarily dissipated as heat (specific dynamic effect), or whether all or part of it is available for physiological work, thus sparing such nutrients as carbohydrates and fats, the food value of which is well known. Confirming many earlier experiments, notably that of Higgins ('17), Bornstein and Loewy ('27) observed no appreciable increase in the basal metabolism of human subjects after the ingestion of 25 to 30 gm. of alcohol by mouth, although rapid absorption of the alcohol was demonstrated by blood analysis, and appreciable oxidation by the observed depression of the respiratory quotient. Somewhat later, Lundsgaard ('31) secured increases as high as 26 per cent in the basal metabolism of dogs by the ingestion of 15 gm. of alcohol. Using rabbits as subjects, Kanai ('33) noted variable effects of alcohol on the basal metabolism, ranging from an initial marked depression followed in 1 or 2 hours by a slight increase, to a prolonged

but slight and irregular increase over a period of 6 hours. Terroine and Bonnet ('29), from a series of experiments on men, rabbits, and pigeons, conclude that alcohol is uniquely and solely thermogenic. If the experiment was performed at high temperatures, from 92 to 97 per cent of the energy of the administered alcohol not appearing in the urine was accounted for by the excess heat produced above the basal metabolic rate. Only if the experiment was performed at temperatures below the critical, was the basal heat production unaffected by alcohol ingestion, a result interpreted as a sparing of body nutrients by the heat of combustion of the alcohol.

The investigations of Mellanby ('19), Onlow ('24), Nicloux ('32), and Widmark ('34), mainly concerned with the rate of disappearance of ingested alcohol from the blood, support the conclusion that this rate is largely independent of the amount of alcohol consumed or of the kinds and proportions of nutrients undergoing simultaneous oxidation. In fact, the recent contributions of Carpenter, Lee and Burdett ('33) and of Nyman and Palmlöv ('34), both upon human subjects, indicate clearly that the rate of disappearance of alcohol from the blood is unaffected by muscular activity (see also Carpenter, '33). Of the same significance are the experimental results reported by Canzanelli, Guild and Rapport ('34), to the effect that the R.Q. of the excess metabolism of work in dogs is unaffected by the ingestion of alcohol, even at times when the alcohol is exerting its characteristic depressing effect upon the R.Q. These investigators conclude definitely that "ethyl alcohol cannot be used as a source of oxidative energy for muscular exercise." However, this conclusion may be a hazardous interpretation of purely negative evidence.

The preponderance of recent experimental work thus appears to favor the view that the energy of ingested alcohol, although completely liberated within the animal body except for insignificant losses through the kidneys (Kionka and Haufe, '28) and lungs (Carpenter, Lee and Burdett, '33) has an extremely limited field of usefulness in metabolism. Shapiro's ('35) experiments afford further evidence of the

same significance, since she showed with rats that ethyl alcohol is distinguished from glucose by its inability to spare body protein during fasting, or to induce a storage of glycogen in the liver, or to exert a ketolytic action following the simultaneous ingestion of diacetic acid, except when a stimulation of protein catabolism occurred; in this case the ketolytic effect was probably exerted by the extra quota of amino acids drawn into the metabolizing mixture.

An attempt to determine the food value of alcohol by feeding experiments upon rats was made by Richter ('26), who found that the rat could tolerate for 3 to 7 months as much as 8 to 16 per cent solutions of alcohol as its steady fluid supply without intoxication, but with a definite decrease in spontaneous activity as measured in revolving cages. The alcohol consumed was estimated to represent 22 to 29 per cent of the total energy intake; the rats consuming these amounts of alcohol ate 17 to 63 per cent less food than the controls which drank water, grew just as rapidly, and reached the same body weight at maturity as the controls. It was concluded that "in the rat alcohol not alone replaces isodynamic quantities of food in maintaining energy balance, as was demonstrated by Atwater and Benedict, but also that it is used for growth and development." Of much the same significance are the observations of Ariyama ('33) that alcohol added to a high fat diet improved the growth rate of rats, the optimum concentration being 5 per cent. On analysis of the carcasses of the rats, it was found that the addition of alcohol to the fat diet favored the deposition of fat in the body, in particular in the liver.

It thus seems difficult to obtain a clear picture of the food value of ethyl alcohol from the evidence available in the literature. Some of the evidence must be discounted because of obvious uncertainties in significance. For example, the failure of Bornstein and Loewy to detect a specific dynamic effect of alcohol in human subjects may have been the result of a failure to place his subjects in the post-absorptive state before administering the drug. The reputedly basal metabolism measurements were made 11 to 13 hours after the last

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The preponderance of recent experimental work thus appears to favor the view that the energy of ingested alcohol, although completely liberated within the animal body except for insignificant losses through the kidneys (Kionka and Haufe, '28) and lungs (Carpenter, Lee and Burdett, '33) has an extremely limited field of usefulness in metabolism. Shapiro's ('35) experiments afford further evidence of the

same significance, since she showed with rats that ethyl alcohol is distinguished from glucose by its inability to spare body protein during fasting, or to induce a storage of glycogen in the liver, or to exert a ketolytic action following the simultaneous ingestion of diacetic acid, except when a stimulation of protein catabolism occurred; in this case the ketolytic effect was probably exerted by the extra quota of amino acids drawn into the metabolizing mixture.

An attempt to determine the food value of alcohol by feeding experiments upon rats was made by Richter ('26), who found that the rat could tolerate for 3 to 7 months as much as 8 to 16 per cent solutions of alcohol as its steady fluid supply without intoxication, but with a definite decrease in spontaneous activity as measured in revolving cages. The alcohol consumed was estimated to represent 22 to 29 per cent of the total energy intake; the rats consuming these amounts of alcohol ate 17 to 63 per cent less food than the controls which drank water, grew just as rapidly, and reached the same body weight at maturity as the controls. It was concluded that "in the rat alcohol not alone replaces isodynamic quantities of food in maintaining energy balance, as was demonstrated by Atwater and Benedict, but also that it is used for growth and development." Of much the same significance are the observations of Ariyama ('33) that alcohol added to a high fat diet improved the growth rate of rats, the optimum concentration being 5 per cent. On analysis of the carcasses of the rats, it was found that the addition of alcohol to the fat diet favored the deposition of fat in the body, in particular in the liver.

It thus seems difficult to obtain a clear picture of the food value of ethyl alcohol from the evidence available in the literature. Some of the evidence must be discounted because of obvious uncertainties in significance. For example, the failure of Bornstein and Loewy to detect a specific dynamic effect of alcohol in human subjects may have been the result of a failure to place his subjects in the post-absorptive state before administering the drug. The reputedly basal metabolism measurements were made 11 to 13 hours after the last

meal, when the R.Q.'s were as high as 0.91 to 0.95. Also Richter's conclusion that alcohol replaces isodynamic quantities of food nutrients in metabolism, takes no account of the observed depression in muscular activity induced by alcohol, nor of the possibility that the gains in weight by test and control rats may have had quite different caloric values. The fact that the experiments were carried out in a room maintained at temperatures ranging from 20° to 22°C., which are considerably lower than the critical temperature of the fasting rat (28° to 30°C.), justifies some suspicion that the alcohol was sparing carbohydrates and fats merely (or in part) because it was providing heat for body temperature maintenance, not because it was furnishing energy for the various forms of physiological work. In Ariyama's experiments, the failure to observe the food intakes of his rats complicates the interpretation of his results with reference to the extent to which alcohol serves as a food. But these, or other, reasonable criticisms are not capable of reconciling all of the apparent conflicts of evidence. The picture is still blurred in many of its aspects.

Evidently there is still a need for further evaluations of ethyl alcohol as a food. The problem is of interest from a number of standpoints, not the least of which is the apparently normal presence of alcohol in body tissues and fluids (Gettler, Niederl and Benedetti-Pichler, '32), and its possible significance as an intermediary metabolite in carbohydrate metabolism (Shapiro, '35). The practical importance of the problem relates to the nutritive evaluation of alcoholic beverages. Attempts at such evaluation must (in the absence of any tenable alternative) ascribe to alcohol, rightly or wrongly, its full energy value of 7.11 calories per gram, and this is done in the medical literature. Thus, Christie ('32) states that "spirituous liquors owe their fat-forming qualities to the alcohol which they contain, and also in some instances to carbohydrate," and he discusses the importance of such beverages in the treatment of obesity and malnutrition.

The investigation reported below is an attempt to furnish further information on the food value of ethyl alcohol, following in general the procedure of Richter, but with certain refinements in technic suggested by a study of his experimental results. The general plan of the experiments has been to feed pairs of rats equal quantities of a good basal diet and to give to one rat of each pair variable supplements of 95 per cent alcohol. The nutritive effects of the alcohol were measured by determining the growth rates of pair mates over a number of weeks of this regime and by investigating the nitrogen and energy contents of the tissues formed from an analysis of the carcasses. The observed effects of alcohol were compared with the nutritive effects of sucrose determined in an entirely similar fashion, supplements of sucrose replacing supplements of alcohol. The comparative effects of alcohol and sucrose supplements upon the digestive processes were also studied by chemical examination of the feces of such paired rats.

To minimize the narcotic effects of the alcohol supplements, they were added to the basal diet rather than to the drinking water as in Richter's experiments. It has been abundantly proven that the rate of absorption of alcohol is definitely depressed by the simultaneous ingestion of food (see for instance, Mellanby, '19). In one series of experiments the rats were placed in revolving cages equipped with revolution counters, so that the alcohol supplements could be increased in size only as long as voluntary activity was not depressed. In a later series of experiments, the rats were confined in every small compartments in the hope of greatly restricting the energy expenditure on muscular activity and of minimizing any differential expenditure between test and control rats. In all experiments the temperature of the room in which the rats were kept was maintained at 80°F. or above in so far as possible. Since the first experiments were performed during warm or hot weather, this object was easily attained, but in the later and, in other respects, more successful feeding experiments, extending into September, it was not possible

to do so consistently, since no temperature control during the night was feasible. However, in the 42 days of this test, the night temperature dropped below 75° only ten times, and below 70° only once. By maintaining temperatures above the critical, it was hoped to distinguish any true nutritive value of the supplement from its ability merely to supply heat to the animal body.

In the first experiments with revolving cages, the alcohol was added to a solid diet, containing 25.5 per cent of whole egg protein and in all other respects capable of promoting rapid growth in rats. While these experiments, involving sixteen pairs of rats, provided clear indications that the energy of alcohol is available for growth, they were unsatisfactory because a variable evaporation of the alcohol from the food dishes undoubtedly occurred. The rats receiving the alcohol supplements were observed soon to defer the consumption of their food until evening, presumably to avoid the alcohol flavor. Under these conditions it was not surprising that in some pairs the growth-promoting effect of the alcohol was not observed. The results are hence statistically inadequate to establish a definite conclusion.

In the final tests, a liquid food, whole fresh milk, was used; the milk was fed in glass water fountains, such as Richter used. One rat in each pair received in its milk an alcohol supplement at the rate of 0.03 cc. per cubic centimeter of milk for the first 9 days of feeding, and then until the termination of the experiment at the rate of 0.04 cc. per cubic centimeter of milk. The alcohol supplement contained 92 per cent by weight of alcohol, 1 cc. having a heat of combustion of 5.29 calories. To prevent the development of anemia, the milk was mineralized by the addition of iron, copper, and manganese salts, according to the directions of Kemmerer, Elvehjem, Hart and Fargo ('32). As thus administered, the surface of alcoholized milk exposed to the air was so small that evaporation of alcohol was minimized, if not prevented for all practical purposes. The volume of milk consumed by pair mates was equalized throughout the test, which lasted 6 weeks.

The activity of the rats was greatly restricted by confining them in small compartments formed by bisecting with a small-mesh screen circular cages measuring only 6 inches in diameter and 7 inches high, each cage thus accommodating a pair of rats. The bottoms of the cages were made of ½-inch mesh wire screening in order to minimize coprophagy.

The rats in the sugar experiment were later fed in the same cages and received also mineralized milk, the consumption of milk by pair mates being also equalized. In this experiment, one rat in each pair received a supplement of sucrose which, except for the first few days, amounted to 2.5 gm. daily, added to the milk in a 50 per cent water solution. The sucrose used contained 3.937 calories per gram by actual combustion in the bomb calorimeter. Since evaporation of volatile substances was a matter of no importance here, the milk and added supplements were offered in open glass dishes held securely in place to prevent spilling. This experiment was continued for 36 days.

The total extra energy consumed by the test rats in these two experiments was nearly the same, averaging 307 calories in the alcohol experiment and 357 calories in the sucrose experiment.

At the termination of each of these experiments the rats were killed with ether, their body lengths from nose to root of tail measured, and the empty weights determined by opening the abdomen and removing the contents of the alimentary tract. The carcasses were then frozen solid and in the frozen condition ground finely in a hand mill. The samples thus prepared were then submitted to chemical analysis. In the alcohol experiments only the nitrogen and gross energy content of the rat carcasses were determined, the former by the Kjeldahl method and the latter by combustion in a Parr oxygen bomb calorimeter. The rats in the sucrose experiments were analyzed for dry matter and ether extract in addition.

The significant results of these two experiments are summarized in tables 1 and 2.

TABLE 1

Results of the alcohol experiment: 42 days

RAT NO. AND SEX	TOTAL MILK CONSUMED	TOTAL ALCOHOL CONSUMED	INITIAL BODY WEIGHT	FINAL BODY WEIGHT	GAIN IN BODY WEIGHT	FINAL BODY LENGTH	EMPTY BODY WEIGHT	COMPOSITION OF EMPTY CARCASS		TOTAL NITROGEN IN CARCASS	TOTAL ENERGY IN CARCASS
								Nitrogen	Gross energy		
	cc.	*cc.*	*gm.*	*gm.*	*gm.*	*mm.*	*gm.*	*per cent*	*cal. per gm.*	*gm.*	*cal.*
77 m	1475	0	43	114	71	181	107	3.14	2.10	3.37	226
78 m	1475	56.8	42	141	99	185	123	3.11	2.59	3.84	320
79 m	1430	0	41	114	73	181	106	3.05	2.19	3.24	232
80 m	1430	55.1	42	144	102	192	135	2.98	2.29	4.04	310
81 f	1335	0	45	99	54	175	92	3.18	2.36	2.92	217
82 f	1335	51.2	39	125	86	182	116	3.00	2.28	3.49	265
83 f	1375	0	36	104	68	173	96	3.24	2.15	3.10	206
84 f	1375	53.2	36	123	87	179	117	2.97	2.29	3.47	268
85 m	1655	0	40	138	98	191	129	3.01	2.29	3.89	295
86 m	1655	63.6	40	160	120	195	152	3.06	2.33	4.64	353
87 m	1825	0	38	158	120	193	147	2.61	2.29	3.83	336
88 m	1825	70.3	38	167	129	198	159	2.93	2.36	4.66	375
89 m	1675	0	37	137	100	189	125	3.00	2.23	3.75	279
90 m	1675	64.1	39	163	124	198	153	3.07	2.36	4.69	361
91 f	1340	0	43	93	50	172	88	3.33	2.01	2.93	177
92 f	1340	50.5	40	116	76	179	108	2.95	2.31	3.18	249

TABLE 2

Results of the sucrose experiment: 36 days

RAT NO. AND SEX	TOTAL MILK CONSUMED	TOTAL SUGAR CONSUMED	INITIAL BODY WEIGHT	FINAL BODY WEIGHT	GAIN IN BODY WEIGHT	FINAL BODY LENGTH	EMPTY BODY WEIGHT	COMPOSITION OF EMPTY CARCASS				TOTAL NITROGEN IN CARCASS	TOTAL ENERGY IN CARCASS
								Moisture	Nitrogen	Fat	Gross energy		
	cc.	*gm.*	*gm.*	*gm.*	*gm.*	*mm.*	*gm.*	*per cent*	*per cent*	*per cent*	*cal. per gm.*	*gm.*	*cal.*
331 m	1215	0	42	123	81	183	113	67.3	3.12	9.35	1.96	3.52	221
332 m	1215	90.7	42	153	111	192	142	64.9	3.02	11.52	2.13	4.30	304
333 f	1582	0	51	114	63	174	107	61.1	3.07	15.52	2.39	3.28	256
334 f	1582	90.7	49	138	89	184	131	59.3	2.96	18.78	2.87	3.89	376
335 m	1850	0	48	145	97	185	134	60.9	3.01	15.82	2.49	4.03	334
336 m	1850	90.7	49	167	118	193	157	62.6	3.00	14.60	2.39	4.71	375
337 m	2110	0	57	165	108	193	153	61.3	3.04	15.36	2.49	4.65	381
338 m	2110	90.7	55	219	164	210	202	61.8	3.04	16.27	2.56	6.12	515
339 f	1757	0	53	129	76	174	119	58.2	2.93	21.31	2.88	3.49	342
340 f	1757	90.7	53	164	111	189	153	62.1	3.00	15.92	2.45	4.61	376
341 f	1765	0	56	120	64	175	113	62.1	2.94	14.57	2.43	3.32	275
342 f	1765	90.7	58	160	102	193	151	60.0	2.95	18.80	2.76	4.45	417
343 m	2055	0	52	168	116	193	152	63.0	3.01	14.42	2.42	4.58	368
344 m	2055	90.7	51	196	145	202	181	59.6	2.80	18.72	2.71	5.07	492
345 f	1491	0	41	111	70	172	101	62.6	3.05	13.97	2.40	3.10	244
346 f	1491	90.7	42	141	99	183	133	58.9	2.96	19.79	2.79	3.94	372
347 m	1642	0	43	135	92	186	121	64.3	3.14	12.57	2.23	3.80	270
348 m	1642	90.7	42	164	122	193	152	63.5	3.07	14.39	2.36	4.66	359

Among the eight pairs of rats in the alcohol test, the rat receiving the alcohol supplement put on the larger live weight gain in all pairs, and attained a greater final empty weight and a greater final body length. From these figures, the growth-promoting value of ethyl alcohol is clearly demonstrated. The percentage of nitrogen in the empty carcasses of control and test rats did not differ in a consistent fashion and averaged very nearly the same, i.e., 3.07 and 3.06. However, the absolute content of nitrogen was greater in all pairs in the carcass of the alcohol rat, the average difference amounting to 0.623 gm. Thus, to this average extent the alcohol supplement promoted nitrogen storage.

The energy content of the carcasses of pair mates, expressed in calories per gram, was greater for the alcohol rat in all pairs but one. A statistical analysis of these paired differences according to the method of 'Student' ('08) indicates a probability of only 0.024 that they were produced by a fortuitous combination of factors common to both pair mates; the average of the differences was 0.149 calorie per gram and their standard deviation, 0.163. Evidently the alcohol produced gains in weight with a greater content of fat than those produced on the basal diet alone. The total excess energy in the carcasses of the alcohol rats, evident in each of the eight pairs, averaged 66.6 calories. This value represents the average recovery of the energy of the alcohol fed in the increase in body substance. For each gram of extra nitrogen stored by the alcohol rats there was an extra deposition of 107 calories of energy.

The sucrose experiment, summarized in table 2, was not designed to demonstrate the food value of sucrose, since this is fully known. Its purpose was twofold, first, to test the method of procedure used in the alcohol experiment with reference to its ability to account for the energy of supplements to the basal diet, and, second, to afford a comparison of the food value of alcohol with that of a common food nutrient of high known value. As would be expected, the rat in each of the nine pairs that received the sucrose supplement

gained in weight more, attained a greater empty weight and body length, and stored more nitrogen and energy in their bodies. The extra nitrogen and energy averaged 0.887 gm. and 99.4 calories, respectively, quantities 42 and 49 per cent greater than the corresponding extra storages observed in the alcohol experiment. The ratio of the two was practically the same as in the alcohol experiment, i.e., 112 calories per gram of nitrogen, as compared with 107.

The carcasses of the sucrose rats contained on the average somewhat less moisture, 61.41 as compared with 62.31 per cent for the controls, somewhat less nitrogen, 2.98 against 3.03 per cent, somewhat more fat, 16.53 against 14.77 per cent, and somewhat more energy per gram, 2.56 against 2.41 calories, but these differences are none of them statistically significant, although the latter two are highly suggestive of an effect of the extra sucrose calories consumed. The probability that chance produced these latter differences are 0.081 and 0.077, respectively.

Before a complete accounting of the extra energy of alcohol and of sucrose provided in the above experiments is possible, it is necessary to consider the effects of these addenda upon the fecal excretions. For this purpose, eight pairs of rats were used. In an initial period the pairs were fed whole milk, the intake of pair mates being equalized. One rat in each pair was given daily 2.5 gm. of sucrose in addition. After a period varying from 11 to 14 days, during which the feces were collected, the sucrose dosage was discontinued, and the control rat was then given a supplement of 2 cc. of ethyl alcohol daily. The collection periods on this regime started from 2 to 9 days later, and continued for 9 to 13 days. The feces were marked by administering to each rat on the first day of each collection period, and also the first day following the termination of the period, a mixture composed of 100 mg. of either Fe_2O_3 or Cr_2O_3 and 1 gm. of glucose. The feces thus collected were dried, sieved to remove the hair, and analyzed for total nitrogen; the heats of combustion were determined in the bomb calorimeter. The results of this test are assembled in tables 3 and 4.

TABLE 3

The effect of sucrose on digestion

RAT NO. AND SEX	AVERAGE BODY WEIGHT	MILK CONSUMED DAILY	SUCROSE CONSUMED DAILY	TOTAL GAINS IN BODY WEIGHT	FECAL CONSTITUENTS EXCRETED DAILY			NITROGEN IN DRIED FECES	ENERGY IN DRIED FECES
					Dry matter	Nitrogen	Gross energy		
	gm.	*cc.*	*gm.*	*gm.*	*mg.*	*mg.*	*cal.*	*per cent*	*cal. per gm.*
383 m	131	40	0	17	351	15.9	1.70	4.52	4.84
384 m	140	40	2.5	38	361	16.8	1.94	4.64	5.37
385 m	130	40	0	16	347	17.2	1.56	4.95	4.50
386 m	144	40	2.5	30	402	21.5	2.12	5.35	5.28
395 m	107	40	0	28	281	13.0	1.31	4.64	4.67
396 m	126	40	2.5	47	368	19.0	2.02	5.16	5.48
397 m	106	40	0	26	327	13.9	1.74	4.26	5.32
398 m	112	40	2.5	37	388	19.9	2.05	5.13	5.28
399 m	115	40	0	16	354	15.6	1.80	4.41	5.08
400 m	131	40	2.5	31	433	22.7	2.46	5.25	5.68
403 f	110	43	0	16	384	19.8	1.81	5.15	4.72
404 f	125	43	2.5	33	344	18.1	1.76	5.26	5.12
409 m	104	40	0	15	344	17.4	1.52	5.07	4.41
410 m	121	40	2.5	35	383	18.5	1.95	4.84	5.09
411 f	101	40	0	17	416	18.7	1.85	4.50	4.44
412 f	107	40	2.5	29	464	25.9	2.32	5.58	5.00

TABLE 4

The effect of alcohol on digestion

RAT NO. AND SEX	AVERAGE BODY WEIGHT	MILK CONSUMED DAILY	ALCOHOL CONSUMED DAILY	TOTAL GAINS IN BODY WEIGHT	FECAL CONSTITUENTS EXCRETED DAILY			NITROGEN IN DRIED FECES	ENERGY IN DRIED FECES
					Dry matter	Nitrogen	Gross energy		
	gm.	*cc.*	*cc.*	*gm.*	*mg.*	*mg.*	*cal.*	*per cent*	*cal. per gm.*
383 m	171	49	2	29	483	24.1	2.24	4.99	4.63
384 m	171	49	0	19	520	24.2	2.53	4.65	4.86
385 m	175	50	2	39	501	27.3	2.28	5.45	4.55
386 m	176	50	0	17	521	25.8	2.56	4.95	4.92
395 m	154	47	2	31	381	18.2	1.82	4.78	4.78
396 m	163	47	0	16	396	18.5	1.94	4.68	4.89
397 m	166	50	2	31	535	26.9	2.92	5.02	5.46
398 m	153	50	0	15	466	22.0	2.32	4.72	4.97
399 m	159	48	2	35	466	22.3	2.36	4.79	5.07
400 m	160	48	0	17	550	26.4	2.77	4.80	5.04
403 f	148	50	2	28	478	27.2	2.13	5.68	4.46
404 f	150	50	0	12	511	26.8	2.39	5.25	4.67
409 m	155	50	2	44	346	17.9	1.52	5.18	4.40
410 m	156	50	0	22	470	22.1	2.23	4.71	4.75
411 f	146	45	2	31	384	23.8	1.73	6.20	4.51
412 f	131	45	0	9	451	24.1	1.96	5.35	4.34

The rats receiving the alcohol or the sucrose supplements gained in weight much faster than their pair mates. However, the effects of the two supplements on the fecal excretion were quite dissimilar. The feces produced on the sucrose supplement, as compared with the control feces, contained significantly larger amounts of dry matter per day (Mean = + 42.4 mg., standard deviation = 38.24, Probability = 0.011), of nitrogen (M = + 3.86 mg., s = 3.13, P = 0.007), and of energy (M = + 0.416 calories, s = 0.235, P = 0.0011), and contained also a significantly greater percentage of nitrogen (M = + 0.464 per cent, s = 0.421, P = 0.011) and a greater energy content per gram (M = + 0.540 calorie, s = 0.252, P = 0.0004). Probably this excess fecal excretion does not represent a depression in the digestibility of the nutrients of the milk, but rather an increased production of metabolic products in the feces. This conclusion is based upon the fact that the ratio of the extra fecal nitrogen (3.86 mg.) to the extra dry matter consumed daily in the sucrose supplement (2.5 gm.), 1.54 mg. per gram, is of the same order of magnitude as the ratio of total fecal nitrogen to consumed dry matter on a diet containing no nitrogen or no indigestible nitrogen (Schneider, '35). The extra fecal energy produced per gram of sucrose supplement is 0.167 calories. The author ('34) has obtained elsewhere an average ratio of 0.164 calorie of fecal energy per gram of a carbohydrate diet consumed; the diet contained no appreciate amount of indigestible nutrients.

In contrast to the sugar, the alcohol supplement was associated with a lower average excretion of fecal dry matter, fecal nitrogen, and fecal energy. However, the average decrease in fecal nitrogen is obviously insignificant, while that of dry matter (M = — 38.9 mg., s = 53.2, P = 0.048) and of energy (M = —0.212 calorie, s = 0.348, P = 0.076) are on the borderline of significance, although in each case only one of the eight pairs presents a contrary result. These apparent effects of alcohol can be interpreted only as an improvement in the digestion of the basal diet of milk, probably brought about by a stimulation of the digestive glands (Newman and

Mehrtens, '32–'33). It is a matter of considerable interest that no evidence of an increased excretion of metabolic fecal products as a result of alcohol ingestion was secured. The percentage of nitrogen in the feces of the rats receiving the alcohol supplements was significantly greater than that of

TABLE 5

The accountable disposition in the animal body of the energy of alcohol

PAIR NO.	EXTRA ENERGY IN ALCOHOL	EXTRA BASAL HEAT	EXTRA ENERGY BALANCE	EXTRA FECAL ENERGY	TOTAL ENERGY OF ALCOHOL ACCOUNTED FOR	
					Calories	Per cent
	cal.	*cal.*	*cal.*	*cal.*		
1	300	58	94	—6	146	49
2	291	56	78	—6	128	44
3	271	23	48	—5	66	24
4	281	37	62	—6	93	33
5	336	36	58	—7	87	26
6	372	53	39	—7	85	23
7	339	54	82	—7	129	38
8	267	20	72	—5	87	33

TABLE 6

The accountable disposition in the animal body of the energy of sucrose

PAIR NO.	EXTRA ENERGY IN SUCROSE	EXTRA BASAL HEAT	EXTRA ENERGY BALANCE	EXTRA FECAL ENERGY	TOTAL ENERGY OF SUCROSE ACCOUNTED FOR	
					Calories	Per cent
	cal.	*cal.*	*cal.*	*cal.*		
1	357	53	83	15	151	42
2	357	45	120	15	180	50
3	357	40	41	15	96	27
4	357	94	134	15	243	68
5	357	81	34	15	130	36
6	357	74	142	15	231	65
7	357	67	124	15	206	58
8	357	50	128	15	193	54
9	357	58	89	15	162	45

the feces of the control rats ($M = +0.372$ per cent, $s = 0.247$, $P = 0.0026$), but obviously the energy per gram was closely the same.

In tables 5 and 6 an attempt is made to account for the extra energy of the alcohol and sugar supplements fed in the

two growth experiments summarized in tables 1 and 2. In the second column of each table will be found the total energy of the supplements. In the next column is an estimate of the extra basal calories of the rats receiving the supplement over that of their controls receiving the same amounts of milk. These values are the summation of values computed weekly from the average weekly weights, the rats having been weighed daily. From the average body weights per week, the average surface areas were calculated by Lee's ('29) formula $S_{cm^2} = 12.54\ W_{gm.}^{0.60}$. The basal metabolism was assumed to equal 760 calories per day per square meter of body surface (Smuts, '35). The extra energy balances, listed in column 4 of each table, represent the extra energy stored in the carcasses of the rats receiving supplements over that of their pair mates, and are computed from the values given in the last columns of tables 1 and 2. The extra fecal energy excreted is a positive value in the case of the sucrose, estimated at 0.167 calorie per gram of sucrose supplement, and a negative value for the alcohol, estimated at — 0.106 calorie per cubic centimeter of alcohol supplement, expressing the fact that sucrose increases the excretion of metabolic fecal products, and the probability that alcohol increases the digestibility of the energy of the basal diet. Adding these various factors together and expressing the sum of the accounted for energy as a percentage of the energy value of the supplement gives the values in the last columns of tables 5 and 6. From 23 to 49 per cent of the energy of the alcohol was thus recovered, the average being 33.8 per cent. For the sucrose the corresponding percentages range from 27 to 68, and average 49.4.

The recovery of the energy of the sucrose supplements thus averaged considerably higher than the recovery of the energy of the alcohol supplements. But individual rats in both experiments exhibited great divergencies from their respective means, so that much over-lapping of individual percentage recoveries may be noted by comparing the values in tables 5 and 6. It becomes a statistical problem, therefore, to deter-

mine whether the sucrose supplements were actually better utilized than the alcohol supplements. Fisher ('28, p. 107) has proposed a modification of the 'Student' method for application to small groups of unpaired observations. Applying this method to the present instance, the mean difference between the alcohol and the sucrose groups is 15.6 per cent, the standard deviation is 11.74, $t = 2.735$, $n = 15$, and $P = <0.02$. This probability that a fortuitous combination of factors common to both groups may have accounted for the average group difference is so small that it may be neglected. We may conclude, therefore, that a more complete recovery of the energy of sucrose was secured than of the energy of alcohol.[1]

The recovery of less than 50 per cent of the energy of the sucrose supplements implies that the specific dynamic effect of sucrose is equivalent to as much as 50 per cent of its total energy content, a seemingly excessive figure. But the average recovery of 49.4 per cent of the sucrose energy is minimal, since the energy expenditure for muscular activity by the sucrose rats would be greater than that of their control mates, assuming the same degree of activity for both, because the sucrose rats were heavier throughout the experiment. This unmeasurable factor is probably of greater importance in the sucrose experiment than in the alcohol experiment, since the sucrose rats gained an average of 6.5 gm. per week more than their control mates, while the alcohol rats gained weekly only 3.9 gm. more than their controls. Also, the presumably greater activity expenditure of the alcohol rats than that of their pair mate controls may well have been entirely nullified if the alcohol supplements depressed the degree of activity on account of their potential narcotic effect. In this connection it must be remembered, first, that muscular activity of all rats was presumably subnormal because of the restricted con-

[1] However, an alternative explanation is that the actual alcohol intake of the rats receiving alcohol supplements was appreciably less than the alcohol offered, due to the evaporation of alcohol from the exposed surface of the milk in the glass fountains. This area equals about 1.75 cm.^{2} It would be extremely difficult to investigate this possibility experimentally. It seems to the writer improbable that this error would be a serious one.

finement, and, second, that the alcohol rats, although observed particularly, never appeared to be apathetic.

It seemed possible that the method of measuring the nutritive energy of sucrose and alcohol by adding them in relatively small amounts to a basal diet possessed a constant error, in the sense that a certain minimum addition would be required before any observable effect at all was obtained. Hence, a series of auxiliary paired-feeding tests were performed with sucrose to determine how large a daily supplement was required to produce a statistically significant effect on body weight and body length with eight pairs during a feeding period of 6 weeks. Daily supplements of 100, 200, and 400 mg. of sucrose were given to one rat in each pair, using a well-balanced diet, and in a later test, the largest supplement was used with a basal diet of mineralized milk.

The rat receiving the 100-mg. supplement of sucrose made the greater gain in weight in only four of the eight pairs, and attained the greater body length in six pairs. The average difference in total gain between pair mates was only 0.75 gm. in favor of the sucrose rats, and the average difference in final body length was only 2.25 mm., again in favor of the sucrose rats. Both differences were obviously insignificant statistically.

The 200-mg. supplement produced even a less decisive result. The test rat gained the faster in four of the eight pairs and attained the greater body length in five of the pairs. While the average differences between pairs favored the sucrose supplement, they were smaller than in the first experiment, i.e., 0.50 gm. and 0.9 mm., respectively.

The 400-mg. supplement to the solid diet was clearly effective in promoting a more rapid growth. In seven of the eight pairs the rat consuming the added sucrose gained the faster and attained the greater body length. The average excess gain of the sucrose rats was 6.5 gm., the standard deviation of differences in gain was 5.87 gm., and the probability of a fortuitous outcome only 0.011. The mean excess body length of the sucrose rat was 2.5 mm., the standard deviation 2.5 mm., and the desired probability only 0.017.

The results of adding a daily supplement of 400 mg. of sucrose to the basal diet of mineralized milk were equally decisive. The mean difference in body weight gain favoring the test treatment was 6.37 gm., the standard deviation of differences, 7.76 gm., and the probability of a fortuitous outcome, 0.034. With reference to the differences in final body length, the statistical results are: $M = 2.62$ mm., $s = 2.45$ mm., and $P = 0.013$.

In this latter test the rats were destroyed and the heats of combustion of their empty carcasses determined. Comparing the energy contents of pair mates, the mean difference was 18.25 calories favoring the test treatment, the standard deviation of differences was 16.88 calories, and the probability of a chance outcome only 0.012.

A reasonable interpretation of these auxiliary experiments is that, in a paired-feeding test involving eight pairs of rats and continuing for 6 weeks, the biological error (measured by the standard deviation of differences in performance between pair mates) is such as to obscure completely the nutritive effect of a daily supplement of 100 and of 200 mg. of sucrose, while it is not sufficient to obscure completely the nutritive effect of 400 mg. of sucrose. It is fair to conclude that the biological error is just large enough to completely obscure the effect of about 300 mg. of sucrose consumed daily. This line of reasoning leads to the expectation that, with a larger number of pairs of experimental animals (and also after a longer feeding period), this obscuring effect of the biological error would diminish until with an infinite number of pairs it would disappear entirely.

If under the conditions of these experiments the nutritive effect of a daily supplement of 300 mg. of sucrose, containing 1.18 calories, is inappreciable, it seems reasonable to assume that the observed nutritive effect of a larger supplement, a grams, containing b calories, would actually be the effect of a —0.3 gm. of sucrose, or of b—1.18 calories from sucrose. Hence, the percentage recoveries of the sucrose calories given in the last column of table 6, may be recomputed by dividing

the total calories accounted for, given in the next to the last column, by 357—((1.18) × 36) = 315 calories, there being 36 days in the experiment. The recomputed percentages average 56, as compared with the uncorrected average of 49.

In making a similar correction of the recovery percentages for alcohol, there is a complication in the fact that the energy of alcohol appears to be less available for physiological purposes than the energy of sucrose, basing this judgment on the average percentage recoveries of 34 for alcohol and 49 for sucrose. If the nutritive effect of a daily supplement of 1.18 calories as sucrose is just obscured by the biological error of the method, it may be expected that a larger calorie supplement in alcohol would exert no appreciable effect. Assessing the daily supplement at $1.18 \times \frac{49}{34}$ and deducting this value, multiplied by 42 (the length of the experiment in days), from the total intake of alcohol calories (table 5, column 2), permits the calculation of first approximations to the correct percentage recoveries. These approximations average 44. A second approximation is obtained by the use of a daily factor of $1.18 \times \frac{56}{44}$, instead of $1.18 \times \frac{49}{34}$. These approximations average 42 per cent.

The specific dynamic effect of sucrose in the rat has not been determined, in so far as the writer is aware, but Kriss, Forbes and Miller ('34) have attempted to determine the heating effect of a starch supplement of 2.2 gm. added to a basal maintenance diet. This effect would presumably approximate the extra heat produced by a supplement of 2.5 gm. of sucrose added to a maintenance diet, but may be considerably less than the specific dynamic effect of the same supplement added to a growth-promoting diet (Forbes, Kriss and Miller, '34). However, in using the data of Kriss, Forbes and Miller, it is necessary to recompute the specific dynamic effects, since all heat production values have been reduced to a constant rat weight of 100 gm., using the ratio of the two-thirds powers of the actual and the standard weights. This so-called 'correction' involves the wholly gratuitous assumption that the specific dynamic effect of a given amount of a nutrient when

fed to rats of different weights varies in direct proportion to the surface areas, the larger rats exhibiting the greater effects. While little or no direct evidence on this point is available one might expect a greater, rather than a smaller, heating effect of a given supplement with the smaller of two rats, because it would represent a higher plane of nutrition (Forbes, Kriss and Miller, '34). In the experiments of Kriss, Forbes and Miller the rats were all lighter when tested with the maintenance ration than when tested with the maintenance ration to which the starch supplement was added, the former weights averaging 100 gm. and the latter 122 gm. The recomputed specific dynamic effect, based upon the assumption that only the basal heat varies with the body surface,[2] the heat increment being unaffected by size of rat, is equivalent to 31.5 per cent of the energy of the starch.[3]

If one may assume on the basis of these results[4] that 2.5 gm. of sucrose added to the maintenance diet of a rat will increase the heat production by an amount equivalent to 31.5 per cent of its energy content, one would expect, on the basis of other

[2] This assumption is used in all of the recent calculations of heat increments of rations for steers by Forbes and his associates. No reason is given for departing from this practice in the rat experiments.

[3] Incidentally when all of the results in this article are recomputed as indicated, the specific dynamic effects of the nutrients studied become:

	Added to a maintenance diet, per cent	*Fed exclusively,*[1] *per cent*
Casein	38.2	30.3
Starch	31.5	20.8
Olive oil	26.2	14.0

[1] Corrected for sparing effect on body protein and fat.

The lack of similarity between these two series of values offers no support for the theory that body nutrients undergoing catabolism exert a specific dynamic effect.

[4] Some hesitation is felt in using even this recomputed specific dynamic effect, because the heat production in all experiments is assumed to be constant throughout the 24 hours. No adequate justification is offered for this practice, concerning which considerable scepticism may reasonably be felt. The average hourly heat production for the day is probably less than the heat production of the 4- or 5-hour experimental periods. It is also possible that the size of the specific dynamic effect is dependent upon the composition of the basal diet.

results from the same laboratory (Forbes, Kriss and Miller, '34), that the same supplement added to a super-maintenance diet may exert even a greater heating effect, since the curve relating heat production and food intake in rats continues a sharp upward turn at levels above the maintenance.

The average disposition within the body of the energy in the sucrose supplements may be pictured as follows. Of the total intake of 357 calories, the effect of 42 calories (12 per cent) was completely obscured by the biological error of the method, the 'extinction quota,' if we may call it such, being equal to 1.18 calories daily. Of the 315 physiologically effective calories, 99 calories (32 per cent) were stored as the new tissues of growth, 62 calories (20 per cent) were expended on the increased basal functions incident to the increased size of body, 15 calories (5 per cent) were, directly or indirectly, represented in the excretion of metabolic fecal products, and 99 calories (31 per cent), or more, were dissipated as heat, constituting the specific dynamic effect. The remaining 40 calories (12 per cent) may have participated in the specific dynamic effect, or they may have been lost in the increased energy expenditure in muscular activity incident to the increased body size.

The average disposition of the alcohol calories followed a somewhat different plan. An average of 307 calories from alcohol were consumed per rat in a total of 42 days. The physiologically ineffective calories (the 'extinction quota') were estimated at 1.59 calories daily, a total of 66 calories (21 per cent). Of the remaining 241 calories, 67 calories (28 per cent) were deposited as the new tissues of growth, 42 calories (17 per cent) were spent on the increment in basal functions consequent upon increased body size, and 6 calories (2 per cent)[5] were gained to the body by the increased digestibility of the basal ration occasioned by the alcohol supplements. The remainder of 138 calories (57 per cent) was probably mainly dissipated as heat.

[5] These 6 calories may be considered as contributing to the energy balance of 67 calories, of which the alcohol can be credited with only 61.

CONCLUSIONS

The energy of ethyl alcohol is to a large extent available for physiological purposes. Added to a complete diet, such as milk, it induces more rapid growth and a greater retention of nitrogen as well as of fat. The new tissue traceable to the alcohol supplement possesses a greater content of fat than that produced on the basal diet alone. The alcohol supplement does not increase the excretion of metabolic products in the feces, but probably does increase the digestibility of the basal diet.

As compared with a similar supplement of sugar, the energy of an alcohol supplement is only about three-fourths as available for physiological purposes, probably because of a greater specific dynamic effect. Its growth-promoting power is definitely less, though the composition of the new tissues produced is similar to that of the gains in weight produced by a sucrose supplement. Its effect on digestion is quite different from that of a sucrose supplement, the latter exerting no appreciable effect on the digestibility of the basal diet, but inducing a greater excretion of metabolic products in the feces in accordance with its content of dry matter.

The biological error in paired-feeding experiments involving eight pairs of rats and continuing for 6 weeks is such that the nutritive effects of a daily supplement of about 1 calorie in the form of sucrose, added to the diet of one rat in each pair, is completely obscured.

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A STUDY OF IRON METABOLISM WITH PRESCHOOL CHILDREN [1]

LEAH ASCHAM

Department of Home Economics, Georgia Experiment Station, Experiment

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Several years ago a dietary regimen was instituted in the preschool cottage of a home for dependent children as a long time feeding experiment. After the adequate diet had been in use for some time an iron utilization study was initiated since data concerning the iron requirements of young children were limited. The results of three investigations have now appeared in the literature. Rose et al. ('30) and Daniels and Wright ('34) propose the equivalent of 0.60 mg. per kilogram of body weight as being adequate to meet the needs of maintenance and growth of children in the preschool group while Leichsenring and Flor ('32) state that 0.48 mg. will provide for a 50 per cent margin of safety. The data reported herein more nearly agree with the higher figure.

EXPERIMENTAL PROCEDURE

Six normal children, four boys and two girls, whose complete cooperation seemed assured, served as subjects for the experiment. Their ages ranged from 4 to 6 years and their weights from 17.1 to 19.3 kg. During the entire experimental period of 19 consecutive days the established schedule of the home was followed in so far as the strict supervision, necessitated by the nature of the study, permitted. This included regularity of meals and in hours of sleep, play out-of-doors, and light chores assigned to the several children. Urine and

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feces, marked with carmine, were collected quantitatively for five periods of 3 days each.

Table 1 lists the weights and average iron content of the different foods served daily.

The children, without exception, consumed all of the food served at each meal and only two adjustments had to be made during the entire time. The portion given each person was the same from day to day with one modification. Bread was the only article in the diet which was designed to fluctuate with the individual desire for bulk and consequently differed

TABLE 1

Average daily intake of iron

FOOD	WEIGHT	AVERAGE IRON CONTENT
	gm.	*mg.*
Cream of Wheat	20	0.34
Rice	25	0.12
Bread	90	1.44
Potatoes	100	0.80
Milk	600	0.58
Cottage cheese	30	0.11
Butter	33	....
Cabbage	30	0.08
Tomatoes (canned)	75	1.09
Turnip greens	200	3.24
Apples (canned)	75	0.58
Pineapple (canned)	50 (slice) 15 (juice)	2.44

with each child, and also varied somewhat daily. Furthermore, it seemed advisable after the eighth experimental day to slightly reduce the amount of turnip greens in order to insure continued success in complete intake of food.

The food for each child was cooked separately in pyrex or new enamel utensils. Butter was the only article which was weighed for a 3-day period, all other supplies were checked daily. An adequate quantity of rice and Cream of Wheat was provided for the entire experiment while a sufficient amount of apples, tomatoes, pineapple and cottage cheese was prepared for each 3-day period. Potatoes were selected from

the same bin. Milk for cooking and drinking was obtained from the home's dairy and turnip greens were picked each morning from special plantings. Bread was delivered daily and cabbage was purchased every third day. Distilled water was used in the preparation of food and by the children ad libitum.

Food for iron analyses was procured as follows:

a. One sample of Cream of Wheat, rice and potatoes representing the entire time.

b. One sample of apples, pineapple, tomatoes, and cheese (two in this instance) made up of aliquots from each period.

c. A composite of daily aliquots of bread, milk, turnip greens, and cabbage for each of the five experimental periods.

Feces and urine were collected by periods. All foods and excreta were kept in glass containers until ready for drying and analysis.

The method for the determination of iron with the details of the technique has been published elsewhere (Ascham, '31).

DISCUSSION

In choosing the diet for this experiment foods low in iron, as shown in the tables of Peterson and Elvehjem ('28), were selected. Except for the iron in canned pineapple which was higher than estimated and that in turnip greens which was lower, the calculated and actual intakes differed by less than 1 mg. for a 3-day period.

No data for the iron content of urine are given. It was concluded, after several analyses, that the influence of the urinary iron upon the final balance would be comparatively insignificant since it was low and relatively constant. Daniels and Wright found 94 to 97 per cent of the iron excretion in the feces, and Rose and co-workers over 95 per cent. Similar low iron output in the urine was reported by Leichsenring and Flor though in their subjects it represented a much higher percentage of the total excretion. According to Marlow and Taylor ('34), the daily urinary iron is also constant in normal adults.

Retentions by periods for two children are given in table 2 and are representative of the group as a whole.

As shown in table 2, H.E. maintained positive balances throughout. During period 4, E.W. had a large negative balance, the largest of any child during the entire experiment; he also had the widest range of retention. Another subject, J.E. maintained a positive balance in all periods, but the remaining three children had two negative balances each. During period 2, D.F. reached a positive balance of 14.39, the largest during the experiment. In only one case, that of E.W., did the highest retention occur in the first period. The four subjects not given in table 2 had the following balances during the first 3 days, —1.38, —1.36, —6.07, and + 3.43.

TABLE 2

Intake, output, and balance of iron by periods

EXPERIMENTAL PERIOD	H. E.			E. W.		
	Intake	Output	Balance	Intake	Output	Balance
	mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
1	30.20	22.37	+ 7.83	28.28	16.27	+ 12.01
2	34.48	33.92	+ 0.56	32.09	32.89	— 0.80
3	31.60	21.76	+ 9.84	30.43	24.66	+ 5.77
4	33.07	29.89	+ 3.18	31.34	41.81	—10.47
5	31.24	24.07	+ 7.17	29.78	22.42	+ 7.36

Their respective highest balances were + 14.39, + 7.14, + 10.20, and + 11.49 which occurred in periods 2, 3, 5, and 3, respectively. Just previous to the first experimental period the medical charts registered hemoglobins of 75 to 82 per cent. It would seem, therefore, that the children had been in fairly good nutritional condition previous to this metabolism study.

The type and number of stools varied with different individuals. There were never less than two passages a day, three or even four were not infrequent, and several times quite soft feces were encountered.

Even with some negative balances, positive retentions were maintained for each subject for the five periods as shown in

table 3 which gives the daily average of the 15 days. It is possible that one or two might have become negative if the urine figures had been included.

The iron requirement as indicated in this study is in fair agreement with the standard of 0.60 mg. per kilogram proposed by Daniels and Wright and by Rose et al. but higher than the allowance of Leichsenring and Flor. The frequent, loose stools, without doubt, had some influence upon the output. Further, it is possible that some of the differences in retention reported by the different investigators might be explained upon the basis of available iron since the diets

TABLE 3

Average daily iron balances for six preschool children

SUBJECT	SEX	WEIGHT	TOTAL IRON			IRON PER KILOGRAM BODY WEIGHT		
			Intake	Output	Balance	Intake	Output	Balance
		kg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
D.F.	Female	18.5	10.56	9.11	1.45	0.57	0.49	0.08
H.E.	Female	19.3	10.71	8.80	1.91	0.55	0.46	0.10
E.L.	Male	17.3	10.91	10.38	0.52	0.63	0.60	0.03
W.H.	Male	19.0	10.74	10.58	0.16	0.57	0.56	0.01
E.W.	Male	17.7	10.13	9.20	0.92	0.57	0.52	0.05
J.E.	Male	17.1	10.98	8.36	2.62	0.64	0.49	0.15

varied widely. Elvehjem, Hart, and Sherman ('33) and Sherman, Elvehjem and Hart ('34 a, '34 b) found the availability of the iron in the materials which they tested ranging from 25 to 100 per cent.

SUMMARY

An iron balance study was conducted for 15 days with six normal children, ages ranging from 4 to 6 years and weights from 17.1 to 19.3 kg.

Iron retentions varied from 0.01 to 0.15 mg. per kilogram with intakes of 0.55 to 0.64 mg. per kilogram, averages 0.07 and 0.59 mg., respectively.

It is assumed that the availability of iron in foods may, in part, account for some of the differences found in the various reports upon the utilization of this mineral by young children.

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RAT'S MILK AND THE STOMACH CONTENTS OF SUCKLING RATS [1]

D. T. MAYER

Laboratory of Biological Chemistry, University of Missouri School of Medicine, Columbia

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INTRODUCTION

In spite of the recognized importance of the role played by the rat as a laboratory animal in nutritional investigation, practically nothing can be found in the literature concerning the character of the diet received by the suckling rat. Some years ago a very brief account of an analysis of the stomach contents of suckling rats was made in a Japanese scientific journal. Our recent efforts to locate this paper have been in vain. Pizzi (1894) included a reference to rat's milk in a paper upon milk of different species of animals. Other than this, we find no indication that the diet of suckling rats has been studied.

Efforts were first directed toward securing rat's milk from the mammary glands of the lactating female rat. However, the arrangement of the mammary glands in a flat, diffuse form makes the securing of the milk a difficult problem, since it prohibits the use of miniature pumps modeled after the human breast pump.

Our next efforts were directed toward a complete analysis of the stomach contents of suckling rats and a study of the conditions existing in their stomachs.

[1] This work has been previously presented in partial fulfillment of the requirements for the degree of master of arts at the University of Missouri.

EXPERIMENTAL

The young rats chosen to supply the milk curd from their stomachs were from 10 to 18 days of age. The latter is about the maximum age at which it is normal to obtain a curd uncontaminated with other food.

The young rats were removed from the cage containing their mother rat at 9 P.M. on the evening before they were to be slaughtered, and kept over night without food or water. At 9 A.M. the next morning they were allowed to suckle as long as they would do so, and then immediately removed, killed by anesthetizing with ether in a closed chamber, and the stomachs dissected out.

An incision was next made in the wall of the stomach from which the contents were allowed to fall into a weighed bottle fitted with a ground glass stopper. Suitable samples were withdrawn from the weighing bottle for the various analyses after the contents had been weighed and thoroughly mixed.

Representative samples of approximately 3 gm. each were withdrawn for the moisture determinations. Drying in an electric oven at atmospheric pressure and a temperature of 90°C. as well as drying in a vacuum oven at 120°C. proved inadvisable since discoloration occurred with loss of water from the sugars in the material. A sample of material dried for 48 hours at 90°C. gave no qualitative test for sugar when tested with Benedict's reagent. Hence it was thought best to place the sample in a vacuum desiccator, then generate the highest vacuum possible with a Cenco oil pump, and allow the sample to dry in this manner until no further change of weight occurred.

Either the Kjeldahl-Gunning or the micro-Kjeldahl method of Cavett ('31) was used for the total nitrogen determinations.

Most of the micro-chemical sugar methods were designed by their authors to apply to glucose. It was found necessary to compare and modify these methods in order to fit them for the determination of lactose. In general, the sugar was determined by agitating a weighed sample of the stomach

contents in 5 to 10 cc. of saturated benzoic acid solution to break the curds, precipitating the proteins by a suitable method, filtering, and then diluting the filtrate to a suitable volume. Portions of the diluted filtrate were then analyzed.

Preliminary tests indicated that although the solutions were already casein-free, the protein precipitation was indispensable. It is a well-known fact that in sugar determinations the pH of the solution is important. Most of the standard methods of protein precipitation gave a more acid filtrate than the standard sugar solution against which it was to be compared. At first these acid filtrates were carefully neutralized, but in the later analyses the proteins were removed by the iron method of Steiner, Urban and West ('32), which gave a neutral filtrate.

A known solution of lactose was hydrolyzed and the resulting sugars determined by the revised Folin-Wu ('29) and the Folin-Malmros ('29) technic. The theoretical amount of galactose and glucose in the hydrolysate was found by both methods when the acid present was neutralized, but errors up to 30 per cent were introduced when no neutralization was made. The above results and the results obtainable by the Folin-Wu method upon cow's milk led us to use it in the majority of the sugar analyses.

The fat was determined by first drying 2- to 3-gm. samples of the stomach contents in vacuo, then extracting for 12 hours with anhydrous ethyl-ether in a Soxhlet extraction apparatus. The ether was removed from the fat by distillation and the fat weighed.

The saponification number of the fat was also determined.

The method of Van Slyke ('23) for chlorides in blood and tissues was adaptable for the determination of chlorides in 1- to 2-gm. portions of the stomach contents.

The pH and titratable acidity were investigated in various samples of the stomach contents.

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RAT'S MILK AND THE STOMACH CONTENTS OF SUCKLING RATS [1]

D. T. MAYER

Laboratory of Biological Chemistry, University of Missouri School of Medicine, Columbia

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INTRODUCTION

In spite of the recognized importance of the role played by the rat as a laboratory animal in nutritional investigation, practically nothing can be found in the literature concerning the character of the diet received by the suckling rat. Some years ago a very brief account of an analysis of the stomach contents of suckling rats was made in a Japanese scientific journal. Our recent efforts to locate this paper have been in vain. Pizzi (1894) included a reference to rat's milk in a paper upon milk of different species of animals. Other than this, we find no indication that the diet of suckling rats has been studied.

Efforts were first directed toward securing rat's milk from the mammary glands of the lactating female rat. However, the arrangement of the mammary glands in a flat, diffuse form makes the securing of the milk a difficult problem, since it prohibits the use of miniature pumps modeled after the human breast pump.

Our next efforts were directed toward a complete analysis of the stomach contents of suckling rats and a study of the conditions existing in their stomachs.

[1] This work has been previously presented in partial fulfillment of the requirements for the degree of master of arts at the University of Missouri.

EXPERIMENTAL

The young rats chosen to supply the milk curd from their stomachs were from 10 to 18 days of age. The latter is about the maximum age at which it is normal to obtain a curd uncontaminated with other food.

The young rats were removed from the cage containing their mother rat at 9 P.M. on the evening before they were to be slaughtered, and kept over night without food or water. At 9 A.M. the next morning they were allowed to suckle as long as they would do so, and then immediately removed, killed by anesthetizing with ether in a closed chamber, and the stomachs dissected out.

An incision was next made in the wall of the stomach from which the contents were allowed to fall into a weighed bottle fitted with a ground glass stopper. Suitable samples were withdrawn from the weighing bottle for the various analyses after the contents had been weighed and thoroughly mixed.

Representative samples of approximately 3 gm. each were withdrawn for the moisture determinations. Drying in an electric oven at atmospheric pressure and a temperature of 90°C. as well as drying in a vacuum oven at 120°C. proved inadvisable since discoloration occurred with loss of water from the sugars in the material. A sample of material dried for 48 hours at 90°C. gave no qualitative test for sugar when tested with Benedict's reagent. Hence it was thought best to place the sample in a vacuum desiccator, then generate the highest vacuum possible with a Cenco oil pump, and allow the sample to dry in this manner until no further change of weight occurred.

Either the Kjeldahl-Gunning or the micro-Kjeldahl method of Cavett ('31) was used for the total nitrogen determinations.

Most of the micro-chemical sugar methods were designed by their authors to apply to glucose. It was found necessary to compare and modify these methods in order to fit them for the determination of lactose. In general, the sugar was determined by agitating a weighed sample of the stomach

contents in 5 to 10 cc. of saturated benzoic acid solution to break the curds, precipitating the proteins by a suitable method, filtering, and then diluting the filtrate to a suitable volume. Portions of the diluted filtrate were then analyzed.

Preliminary tests indicated that although the solutions were already casein-free, the protein precipitation was indispensable. It is a well-known fact that in sugar determinations the pH of the solution is important. Most of the standard methods of protein precipitation gave a more acid filtrate than the standard sugar solution against which it was to be compared. At first these acid filtrates were carefully neutralized, but in the later analyses the proteins were removed by the iron method of Steiner, Urban and West ('32), which gave a neutral filtrate.

A known solution of lactose was hydrolyzed and the resulting sugars determined by the revised Folin-Wu ('29) and the Folin-Malmros ('29) technic. The theoretical amount of galactose and glucose in the hydrolysate was found by both methods when the acid present was neutralized, but errors up to 30 per cent were introduced when no neutralization was made. The above results and the results obtainable by the Folin-Wu method upon cow's milk led us to use it in the majority of the sugar analyses.

The fat was determined by first drying 2- to 3-gm. samples of the stomach contents in vacuo, then extracting for 12 hours with anhydrous ethyl-ether in a Soxhlet extraction apparatus. The ether was removed from the fat by distillation and the fat weighed.

The saponification number of the fat was also determined.

The method of Van Slyke ('23) for chlorides in blood and tissues was adaptable for the determination of chlorides in 1- to 2-gm. portions of the stomach contents.

The pH and titratable acidity were investigated in various samples of the stomach contents.

RESULTS

Weights of the stomach contents

A characteristic variation in the weights of the stomach contents was noted. A steady increase in stomach content weight up to the twelfth day was followed by a more rapid increase in weight for the next 7 days. At the seventh day the average weight of the contents was but 0.197 gm., at the twelfth day they weighed 0.8086, and at the nineteenth day the weight had increased to 1.5903 gm.

TABLE 1

Protein analyses

SAMPLE NUMBER	SOLUBLE NON-PROTEIN NITROGEN, PER CENT		SOLUBLE PROTEIN NITROGEN, PER CENT		SOLUBLE PROTEIN, PER CENT		TOTAL, NITROGEN, PER CENT		TOTAL PROTEIN PER CENT WET BASIS
	Wet basis	Dry basis	Wet basis	Dry basis	Wet basis	Dry basis	Wet basis	Dry basis	
I Dry weight = 22.2 per cent	0.4406	1.99	0.4664	2.10	2.99	13.4	1.51	6.81	6.89
II Dry weight = 22.2 per cent	0.437	1.97	0.470	2.12	3.00	13.5	1.509	6.80	6.89
III Dry weight = 22.2 per cent	0.438	1.98	0.469	2.11	3.00	13.5	....	....	....

Appearance of the stomachs

The appearance of the stomachs proved to have an important bearing upon the desirability of the contents for the analyses. Usually the stomachs were fully distended. When newly filled, they had a milk-white appearance. At later stages the digested portions were an amber color and were usually found near the pyloric end of the stomach, where the digestion unquestionably first began. If food, other than milk had been ingested, it could easily be detected. In most cases the curds were well-formed. Occasionally, however, curd

TABLE 2

Sugar and fat analyses

SUGAR			FAT			
Sugar method used	Per cent lactose in sample	Method of protein precipitation	Fat per cent: Wet basis	Fat per cent: Dry basis	Saponification number of fat	Molecular weight calculated from average saponification number
1. Folin-Malmros neutralized	3.41	H_2SO_4-Na-tungstate	12.4	55.8	208.6	
2. Folin-Malmros neutralized	3.36	Hcl-tungstic acid (7 per cent Hcl)	12.4	55.8	211.6	
3. Folin-Malmros neutralized	3.38	Hcl-tungstic acid (7 per cent Hcl)	12.4	55.8	210.8	
4. Folin-Wu	3.37	Folin's blood method	12.4	55.8	210.6	
5. Folin-Wu	3.37	$Fe_2(SO_4)_3$-$BaCO_3$	12.4	55.8	210.6 (average)	801.1
6. Folin-Wu	3.39	$Fe_2(SO_4)_3$-$BaCO_3$	....	....	....	
7. Folin-Malmros	3.42	$Fe_2(SO_4)_3$-$BaCO_3$	....	....	....	

[1] Moisture of all samples = 77.8 per cent, dry weight = 22.2 per cent.

TABLE 3

SAMPLE AND DRY WEIGHT	TOTAL CHLORIDES AS Nacl PER CENT		TOTAL CHLORIDES AS Hcl PER CENT		TITRATABLE ACIDITY CUBIC CENTIMETERS OF N/10 NaoH PER 100 GM. OF CONTENTS	ACIDITY EXPRESSED AS PER CENT BY WEIGHT OF Hcl IN CONTENTS		HYDROGEN ION CONCENTION EXPRESSED AS pH
	Wet basis	Dry basis	Wet basis	Dry basis		Wet basis	Dry basis	
22.2	0.234	0.778	0.146	0.498	83.9	0.306	0.908	5.8
22.2	0.234	0.779	0.146	0.498	83.85	0.30	0.90	
22.2	0.234	0.779	0.146	0.498	83.9	0.306	0.908	

TABLE 4

Complete analyses of the sample of stomach contents which most closely approached normal rat's milk as indicated by lack of curd formation

MOISTURE, PER CENT	PROTEIN, PER CENT	CARBOHYDRATE (SUGAR), PER CENT	FAT, PER CENT	CHLORIDES (AS Hcl), PER CENT
77.84	6.89	3.36–3.42	12.4	0.146

formation had progressed but little, so that the contents were semi-fluid in character. The semi-fluid contents were the most desirable for analyses.

Dry weight determinations

The dry weight of the contents varied from 22.2 per cent to 45.3 per cent, depending upon the time between suckling and the securing of the samples. Samples secured immediately after suckling were semi-fluid, hence were more representative of a normal milk. The semi-fluid contents had a moisture of 77.8 per cent or a dry weight of 22.2 per cent.

Evidence, as yet not fully substantiated, is indicative of a low tryptophane-containing casein for rat's milk.

DISCUSSION

The percentage of solids present in the stomach contents was dependent upon the time that the milk remained in the stomachs—the moisture decreasing as the time increased. As the curd formed, fluid was expressed from the curdling mass and it soon passed out of the stomach. Before this process the percentage of dry weight is of a similar order of magnitude to various other milks although greater than the average of milks that have been reported upon.

The very slight variation in the percentage of fat calculated upon the basis of the dry weight indicates the lack of a significant quantitative change in the fat while in the stomach.

The sugar, chlorides, and soluble protein are dependent upon the quantity of water present. Since they are all soluble in water, they are undoubtedly removed in the fluid expressed from the curd and passed with the fluid into the intestines. The hydrochloric acid of the gastric juices increases the total chloride values.

The proteins of the stomach contents might vary slightly. The digestive juices may add varying quantities of protein and a slight additional variation through protein digestion is possible. But as already stated, digestion is easily detected by a color change in the contents from white to amber, and

analyses of partially digested samples were excluded from the tables.

In spite of the possible slight variations in the analyses of the stomach contents, these analyses do give relative values; and, in most cases, these values are a fair indication of what one should expect for the normal milk.

Those analyses which most nearly represent the original, unmodified milk, indicate about 22.2 per cent solids, of which the average values are: for fat 55.8 per cent, for proteins 31.03 per cent, and for carbohydrates 15 per cent.

Three milks—cow's, goat's and human—are widely used in human nutrition, especially infant nutrition. From the standpoint of nutritional interest a comparison of these milks with rat's milk should be of value.

Rat's milk contains six times the protein of human milk and about three times that of either cow's or goat's milk.

The fat of rat's milk is about four times that of either human or cow's milk. Animals mentioned in the literature as having a milk of exceptionally high fat content are large in most cases. It is a notable fact that large ocean-living mammals have a very high percentage of fat in their milks, especially the dolphin with 43.46 per cent, Hammarsten ('10). Of the smaller animals mentioned in the literature, the milks of the dog, cat and rabbit, Folin, et al. ('19), show the highest percentage of fat.

SUMMARY

The analyses reported of protein, fat and sugar indicate a high fat and a low carbohydrate diet for the suckling rat.

Fats comprise approximately 55 per cent of the solids in rat's milk, approximately 30 per cent are proteins, and approximately 15 per cent are carbohydrate.

Calculating the proportion of calories upon the basis that fat yields 9.3, protein about 4, and disaccharides 3.95 calories per gram, 74 per cent of the calories in the diet of the suckling rat are furnished by fat, 18 per cent by protein, and only 8 per cent by carbohydrate.

In spite of the great proportion of fat in the diet of the suckling rat, this diet is not an extremely ketogenic one. The ketogenic-antiketogenic ratio, calculated as suggested by Shaffer ('21 a, '21 b, '22), is 1.35 : 1.

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